

Glyphosate resistance in *Lolium rigidum* Gaud. in Australia

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Summary Glyphosate is a widely used non-selective herbicide in Australia. The intensive use of glyphosate has resulted in the evolution of glyphosate resistance in *Lolium rigidum*. Several populations of *L. rigidum* from around Australia are known to be resistant to glyphosate. There are variations between some of these populations in their response to glyphosate suggesting there may be differences in the mechanism of resistance between populations. Preliminary experiments indicate that resistance in three different populations can be attributed to a nuclear inherited dominant trait. Examination of glyphosate translocation patterns in two of these populations indicates they share a similar resistance mechanism.

Keywords *Lolium rigidum*, glyphosate resistance, genetics, resistance mechanism.

INTRODUCTION

Glyphosate is a major non-selective herbicide used for control of weeds in many situations. It is used to control of weeds prior to seeding in grain crops, within rows of tree and vine crops, in fallows, along roadsides, railways, and other industrial uses (Dyer 1994). Resistance to glyphosate was first observed in 1996 in one population of annual ryegrass (*Lolium rigidum* Gaud.) (Pratley *et al.* 1996) in Australia, and has since been reported in other populations of this weed species (Powles *et al.* 1998). To date, there are eleven known glyphosate-resistant populations of *L. rigidum* in Australia. Long periods, often 15 years or more, of exposure to glyphosate are common to all resistant populations, which have predominantly been associated with zero-tillage grain cropping and perennial horticulture systems.

Glyphosate resistance in the population (NLR 70) first reported by Powles *et al.* (1998) is conferred by a single nuclear gene inherited in a semi-dominant manner (Lorraine-Colwill *et al.* 2001). Here we present data from dose response experiments of several other resistant populations of *L. rigidum* collected from various sites around Australia. Initial F₁ inheritance data from two populations, VLR 15 and SLR 76, is also presented, along with preliminary results of glyphosate translocation patterns in SLR 76.

MATERIALS AND METHODS

Dose response of putative resistant (r) populations Seed from four putative resistant (R) populations were tested for their dose response to glyphosate isopropylamine. All populations came from areas where glyphosate has been intensively used over a long period of time. NLR 71 originated from a winter fallow, summer crop rotation on the Liverpool Plains of NSW, and NLR 72, also from a winter fallow, summer crop rotation, came from Baradine, NSW. SLR 76 was collected from a vineyard at McLaren Vale, SA, while VLR 15 was collected from a grain cropping farm near St. Arnaud, Victoria. A known susceptible population (VLR 1) and a known glyphosate resistant population (NLR 70) were also used.

Dose responses of these populations were performed in two separate experiments. The first examined NLR 71 and NLR72, and the second VLR 15 and SLR 76. Both experiments contained the known susceptible (S) and resistant (NLR 70) populations, which were included as controls. Seed of all populations was germinated as described by Lorraine-Colwill *et al.* (2001). Seedlings were transferred to 17 cm pots (12 seedlings per pot, 20 pots per population) containing standard potting mix and maintained outdoors during June and July of 2001. Once seedlings had reached the two to three leaf stage, glyphosate isopropylamine with 0.2% by volume non-ionic surfactant was applied at rates from 0 to 3600 g a.e. ha⁻¹. Herbicide was applied using a laboratory moving boom sprayer equipped with T-jet fan nozzles at a speed of 1 m s⁻¹. The output volume from the sprayer was 128 L ha⁻¹ at a pressure of 250 kPa. Plants were returned outdoors after treatment and the response to the herbicide treatment recorded after 21 days. Plants were recorded as alive if they had strongly tillered since application of the herbicide.

LD_{50,s}, the concentration of glyphosate required for 50% mortality, and standard errors (SE) of each population were calculated by probit analysis.

Creation and dose response of F₁ families Seedlings of twice selected VLR 15 and SLR 76 were crossed with a known susceptible population (VLR 1) as

described by Lorraine-Colwill *et al.* (2001). Parental populations of both R and S populations were selected to be homozygous for resistance or susceptibility (data not shown). Seeds were harvested (December 2000) from both R and S parents and stored separately to constitute individual F₁ families.

Seeds from six VLR 15 and SLR 76 F₁ families, from both R and S maternal plants were germinated as described previously, along with S and R parental controls. Plants were transferred to pots situated outdoors and allowed to grow until tillering. Tillering plants (12 per family or population) were divided into three segments of two tillers and potted into two-inch square pots (one segment per pot) and grown in a heated and cooled glasshouse (September 2001). Once plants had grown another 2 to 3 cm of fresh leaf material, they were treated with glyphosate isopropylamine containing 0.2% non-ionic surfactant as described above, at rates of 337.5, 450, and 562.5 g a.e. ha⁻¹. Plants were returned outside after spraying and the response to the herbicide treatment recorded after 21 days.

Translocation of ¹⁴C-glyphosate in *L. rigidum*

L. rigidum seeds were germinated and grown hydroponically by the method described by Lorraine-Colwill *et al.* (2002) (in press). Glyphosate, ¹⁴C-labelled at the phosphonomethylene (C-2) site was purchased from Sigma-Aldrich. The radiolabelled solution of glyphosate used contained ¹⁴C-glyphosate (1.4 μmols) diluted with a solution of glyphosate isopropylamine (5.41 μmols) and non-ionic surfactant (0.2% v/v Wetter TX), and stored at -20°C. Application of radiolabelled glyphosate, plant harvest, and radioactivity determinations were carried out as described by Lorraine-Colwill *et al.* (2002). Plants were analysed in three parts. These were the treated leaf, including the application site, the rest of the shoot and the roots.

RESULTS AND DISCUSSION

The S population of *L. rigidum* was well controlled by the 450 g a.e. ha⁻¹ of glyphosate (Figure 1). In contrast, the known resistant population (NLR 70) required higher doses of glyphosate for significant mortality to occur. Dose response data for the putative resistant populations NLR 71 and NLR 72 (Figure 1) showed these populations responded in a very similar manner to resistant population NLR 70. Significantly more glyphosate was required to control these populations than the susceptible population VLR 1. This provides confirmation that these two populations are resistant to glyphosate. Considerable mortality was observed for all populations in this experiment. Extremely cold conditions were experienced before and after herbicide treatment. This dose response experiment

was repeated in October 2001 (data not shown) with similar trends.

In the second dose response experiment, the control S and R populations showed similar responses to glyphosate (Figure 2) as that observed for these populations in the first experiment (Figure 1). The putative resistant populations VLR 15 and SLR 76 behaved more similarly to the R population than the S population (Figure 2), indicating that these populations are also resistant to glyphosate. Population SLR 76 was more tolerant of glyphosate than VLR 15.

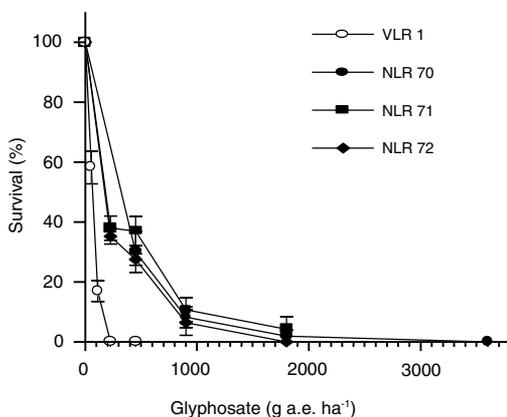


Figure 1. Survival of the known susceptible (VLR 1), known resistant (NLR 70), and putative resistant (NLR 71 and NLR 72) populations of *L. rigidum* to various dose rates of glyphosate isopropylamine. Data are from a single dose response experiment with four replicate pots of seedlings treated at each dose. Points are mean survival ± SE.

Table 1. LD₅₀, concentration of herbicide required for 50% mortality, of the putative resistant populations of *L. rigidum*.

Population	LD ₅₀ (g a.e. ha ⁻¹)	SE
Experiment 1		
VLR 1	71.63	5.38
NLR 70	429.9	51.7
NLR 71	362.7	57.3
NLR 72	293.0	32.2
Experiment 2		
VLR 1	45.63	4.63
NLR 70	246.6	18.9
VLR 15	215.4	16.8
SLR 76	459.1	46.7

The LD₅₀ for glyphosate, the concentration of herbicide required for 50% mortality, of the putative resistant populations ranged from 215.4 to 459.1 g a.e. ha⁻¹ (Table 1). SLR 76 had the highest LD₅₀ of all populations tested, with VLR 15 having the lowest of

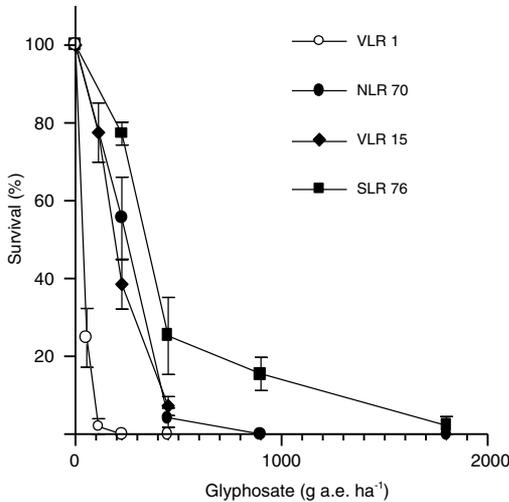


Figure 2. Survival of the known susceptible (VLR 1), known resistant (NLR 70), and putative resistant (VLR 15 and SLR 76) populations of *L. rigidum* to various dose rates of glyphosate isopropylamine. Data are from a single dose response experiment with four replicate pots of seedlings treated at each dose. Points are mean survival \pm SE.

the resistant populations (Table 1). These differences in LD₅₀s could reflect differences in herbicide resistance mechanisms between these populations.

F₁ dose response of VLR 15 and SLR 76. Most plants of the resistant parental populations VLR 15 and SLR 76 survived each of the three doses of glyphosate (Figure 3). In contrast, most of the susceptible (VLR 1) plants were controlled by 553 g a.e. ha⁻¹ (Figure 3). Response of the F₁ families was in all cases very similar to their resistant parents and very different from that of the susceptible parent. Therefore, glyphosate resistance in both these populations appears to be inherited in a largely dominant manner. Responses of the seed from maternal S or maternal R populations were similar (Figure 3), indicating the resistance trait is not inherited maternally, but inherited on the nuclear genome. Glyphosate resistance in NLR 70 is also inherited as a nuclear gene in a largely dominant manner (Lorraine-Colwill *et al.* 2001).

Translocation of glyphosate An experiment was carried out on the resistant population SLR76, to compare the movement of glyphosate in this population to that of the resistant population NLR 70 and the susceptible VLR 1. Glyphosate was readily and rapidly absorbed into treated seedlings, with an average of 59% of applied glyphosate absorbed two hours after treatment (HAT). In the susceptible plants, glyphosate moved out of the treated leaf over time with a third of the herbicide accumulating in the roots 24 HAT

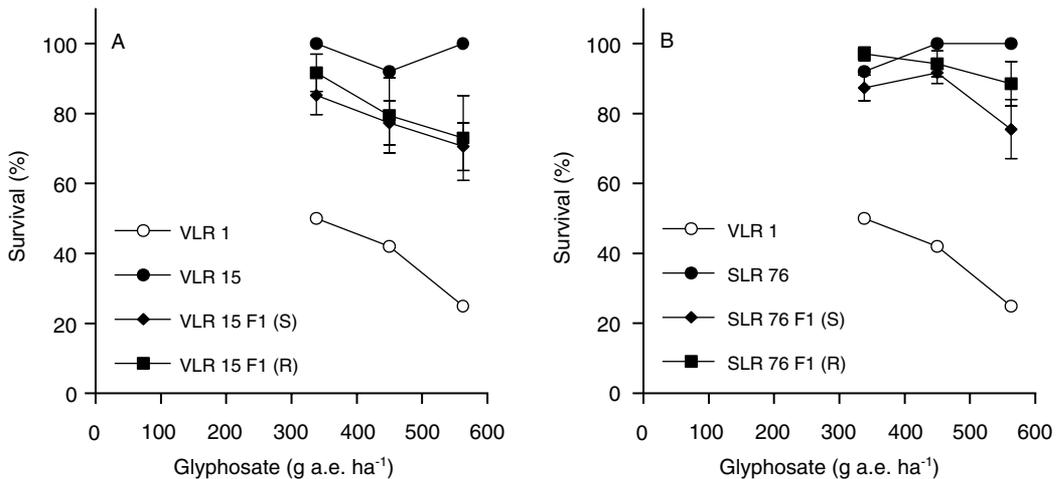


Figure 3. Dose response of S, R, and F₁ clones of *L. rigidum* from crosses between susceptible VLR 1 and resistant VLR 15 (A) or SLR 76 (B) populations. Data are from a single dose response experiment with 12 plants per family split into three clones tested at three glyphosate concentrations. Data points for R and S parents represent the mean of 12 plants, and for the F₁ the mean of 72 plants (six families per F₁ cross). Error bars are SE.

(Table 2). The pattern of glyphosate translocation within plants of the two R populations was somewhat different (Table 2). More herbicide tended to remain within the treated leaf and less was translocated to the roots. At 24 HAT, markedly less glyphosate was present in roots of plants of either resistant population compared to the susceptible population.

The pattern of glyphosate translocation in SLR 76 was more like that of NLR 70 than the susceptible VLR 1. This similarity in response suggests that SLR 76 may have the same mechanism of resistance as NLR 70. The pattern of translocation away from the roots reported here in R plants is similar to that reported by Lorraine-Colwill *et al.* (in press), where resistance in NLR 70 was correlated with reduced translocation of glyphosate to the roots.

An increasing number of glyphosate resistant populations of *L. rigidum* are being documented in Australia. These populations come from a variety of systems including grain cropping, vineyards, orchards, and winter fallows. The experiments reported here compare the dose responses of five glyphosate-resistant *L. rigidum* populations. There are some differences in dose response between populations raising the possibility that different mechanisms of resistance to glyphosate may occur. However, in two resistant populations, glyphosate movement, particularly to the roots seems to be restricted. In three resistant populations, glyphosate resistance is inherited as a largely dominant nuclear gene (Lorraine-Colwill *et al.* 2001). Similarities between the dose responses and glyphosate translocation in these populations suggest that they may all share the same mechanism of resistance. If this were the case, it would greatly simplify the development of methods to control resistant *L. rigidum* in Australia.

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Table 2. Translocation of ^{14}C glyphosate in S (VLR 1) and R (NLR 70 and SLR 76) *L. rigidum* plants. Values shown are averages of 3 to 5 plants per population for each time point \pm SE.

HAT	Population	^{14}C detected (% of total absorbed)		
		Treated leaf	Rest of shoot	Root
2	VLR 1	36 \pm 5	55 \pm 5	9 \pm 1
	NLR 70	33 \pm 7	64 \pm 7	3 \pm 1
	SLR 76	56 \pm 5	40 \pm 5	4 \pm 1
24	VLR 1	22 \pm 6	49 \pm 7	29 \pm 3
	NLR 70	35 \pm 5	51 \pm 5	17 \pm 4
	SLR 76	31 \pm 3	52 \pm 1	17 \pm 3

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