The cost of glyphosate resistance: is there a fitness penalty associated with glyphosate resistance in annual ryegrass?

Angela M. Wakelin1 and Christopher Preston1,2

1 School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, PMB 1, Glen Osmond, South Australia 5064, Australia
2 CRC for Australian Weed Management, PMB 1, Waite Campus, South Australia 5064, Australia

Summary  Glyphosate is a broad spectrum herbicide that is a critical weed control tool in Australia for agricultural, horticultural, and viticultural farming systems. The long term use of glyphosate worldwide has led to the evolution of resistance in nine different weed species. In Australia at least 54 populations of annual ryegrass (*Lolium rigidum* Gaudin) have been confirmed resistant to glyphosate. Many of these resistant populations appear to have altered translocation of glyphosate within the plants. We have investigated whether this mechanism of resistance causes a reduction in fitness in resistant individuals in a competitive field crop situation. F2 seedlings of four different glyphosate resistant annual ryegrass populations from NSW and SA were planted in a wheat crop in 2003 and allowed to cross only within each F2 family by weeding all wild ryegrass from the trial. Seed was collected at the end of the 2003 season, and seedlings from this seed planted back in the same plots the following season. The frequency of glyphosate resistance was determined in the original F2 seed, and the seed collected in 2003 and 2004 to determine the change in frequency of glyphosate resistance over time in the absence of selection pressure by glyphosate. In all four populations the frequency of glyphosate resistant individuals decreased by 40% in the first year and 20% in the second year.

Keywords  *Lolium rigidum*, fitness, glyphosate resistance.

INTRODUCTION
Glyphosate is the active ingredient in the world’s most widely used broad-spectrum herbicide, Roundup™. In Australia, it is used in a variety of situations, including broad-acre cropping, horticulture, and viticulture, in fallows and along fence lines and drainage ditches (Dyer 1994). Glyphosate was used in Australia for many years before the first reports of resistance in annual ryegrass (*Lolium rigidum* Gaudin) in 1995 (Powles et al. 1998, Pratley et al. 1999). The incidence of glyphosate resistance in annual ryegrass has continued to increase, with 54 confirmed glyphosate resistance populations to date from Western Australia, South Australia, Victoria and New South Wales (Preston 2006). An important issue in the management of glyphosate resistance is whether the presence of the glyphosate resistance allele is associated with a physiological disadvantage to the plant in the absence of the herbicide. This cost is also an important factor in modelling calculations aimed at modelling the population dynamics of herbicide resistance evolution (Vila-Aiub et al. in press). There are several important considerations in terms of methodology for fitness experiments. In order to control for the effects of genetic background on fitness, lines used should be as homogeneous as possible (Bergelson and Purrington 1996). This is usually obtained by creating near isogenic lines of S and R plants, or can occur through crossing R and S lines to create populations segregating for resistance (F2) effectively randomising the effects of background genes.

Creating a perfectly homogeneous genetic background is only possible via the use of transgenic plants, where one can be sure that the only difference between R and S lines is the presence of the resistance gene, e.g. Purrington and Bergelson (1997). There is also evidence that resource limitation is an important factor in determination of fitness. Bergelson (1994) showed that the costs of resistance in a lettuce cultivar resistant to leaf root aphid and downy mildew were enhanced in plants grown under low nutrient conditions.

The mechanism of glyphosate resistance has been studied in several populations, providing evidence for both target-site (Wakelin and Preston in press) and non-target-site (Baerson et al. 2002, Lorraine-Colwill et al. 2003, Wakelin et al. 2004) mechanisms of resistance. Initial studies into the fitness cost of glyphosate resistance were performed on a resistant biotype from New South Wales, NLR 70. This work found that this biotype was subject to a significant fitness penalty due to the presence of the glyphosate resistance allele (Neve et al. 2002). Here we present the results of an experiment that continues the investigation into the effects of glyphosate resistance on plant fitness. We placed F2 seedlings of four glyphosate-resistant annual ryegrass populations into a competitive situation with a wheat crop, in the field, over two years, and measured the change in frequency of glyphosate resistant individuals in the absence of selection with glyphosate.
MATERIALS AND METHODS

Plant material Populations selected for this experiment originated from New South Wales and South Australia (Table 1). The mechanism of resistance in all four populations is related to differential translocation of glyphosate (Lorraine-Colwill et al. 2003, Wakelin et al. 2004), and all have resistance attributable to a single, partially or fully dominant, nuclear gene (Wakelin and Preston 2006).

Creation of F2 families Glyphosate-resistant (R) individuals that were survivors of a dose response experiment of bulked seed were crossed with susceptible (S) individuals (population VLR 1) as described by Lorraine-Colwill et al. (2001) to create F1 families. Families were confirmed as true F1 via a dose response experiment (Wakelin and Preston 2006). F2 families were created by crossing four F1 individuals from confirmed F1 families together in one pot and allowing them to set seed. F2 seed was germinated on 0.6% agar in a controlled environment cabinet (12 h light/dark cycle, 20/15°C) for one week before transferring seedlings to potting mix in 6-cell trays. Timing of planting seeds was determined by the season break each year.

Field experiment design The field site was located at the Roseworthy Campus of the University of Adelaide, Australia. A field was selected that was to be cropped for the duration of the trial, in order to allow competition of the ryegrass with crop plants. The crop in 2003 was wheat (Triticum aestivum L.), and in 2004 was common vetch (Vicia sativa ssp. sativa L.). A half hectare area was marked out prior to crop seeding in 2003, and 16 plots of 1 × 2 m size were placed in a grid pattern within the half hectare, 10 m apart. Families were randomly assigned to the plots, with four F2 families from each population used as replicate plots. Seedlings were planted at a density of 100 plants m⁻², and identified by placing a curtain ring around the base of each plant.

Table 1. Geographic source and glyphosate resistance status of L. rigidum populations used in this study.

<table>
<thead>
<tr>
<th>Population</th>
<th>Location</th>
<th>Resistance level⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLR 1 (S)</td>
<td>Serviceton, Vic</td>
<td>–</td>
</tr>
<tr>
<td>NLR 70</td>
<td>Orange, NSW</td>
<td>6.0</td>
</tr>
<tr>
<td>NLR 71</td>
<td>Bundella, NSW</td>
<td>5.1</td>
</tr>
<tr>
<td>NLR 72</td>
<td>Baradine, NSW</td>
<td>4.1</td>
</tr>
<tr>
<td>SLR 76</td>
<td>McLaren Vale, SA</td>
<td>10.1</td>
</tr>
</tbody>
</table>

⁴ Resistance level was calculated from data in Wakelin et al. (2004) as the ratio of LD₅₀ₗₘₜ of resistant populations and the susceptible (VLR 1) population.

RESULTS

Total seed collected from three of the plots from the first harvest in 2003 was low and therefore all seed was sown the following season from these plots to grow enough seedlings for planting. This meant that there was not enough seed remaining from these plots from the 2003 harvest at the end of the experiment for frequency testing. This caused the data to be unbalanced when analysed via a two-way ANOVA.

No significant interaction was observed between the population and year (P = 0.7216) nor between the populations (P = 0.3899). A significant effect was
attributed to the year of harvest ($P = 0.0224$). This effect of year is shown by the decline in frequency of glyphosate-resistant individuals in Figure 1. In the first year of seed set in the absence of glyphosate (2003 seed), the percentage of glyphosate-resistant individuals dropped by approximately 40%, while in the second year (2004) the decline was only 20% (Figure 1). The survival of the parental bulked populations varied from zero (S population) to 91.9% survival (SLR 76) (Table 2).

DISCUSSION

Two critical factors in the experimental design of fitness experiments are that the genetic backgrounds of the individuals be as similar as possible, and that the plants be grown in a stressful situation, to increase the likelihood of detecting the fitness cost (Bergelson 1994, Bergelson and Purrington 1996). This experiment fulfilled both of these factors, with the use of $F_2$ families and by planting them in the field in competition with a crop.

This experiment detected a significant fitness cost associated with glyphosate-resistance that resulted in a reduction in the frequency of glyphosate-resistant individuals over time (Figure 1) in the absence of glyphosate use. This fitness cost was observed in all four glyphosate-resistant populations studied. This result is similar to the findings of Neve et al. (2002) who presented results of a fitness penalty present in the glyphosate-resistant population NLR 70.

Studies on the effect of herbicide resistance on fitness in other species have found conflicting results (reviewed by Vila-Aiub et al. (in press). A fitness effect has been reported for resistance to acetyl-CoA carboxylase (ACCase) herbicides (Vila-Aiub et al. 2005), but there are also reports of no fitness effect associated with this type of herbicide resistance (Holt and Thill 1994). Vila-Aiub et al. (2005) emphasised that it is important to definitively study each mutation or resistance mechanism as each genetic change is likely to affect fitness differently. We studied the change in resistance frequency in four populations with a similar mechanism of resistance (translocation). Because the mutations involved in this type of non-target site resistance are unknown, we cannot say whether this fitness effect will be exhibited in all other *L. rigidum* populations that share this mechanism. There may be multiple mutations that confer this type of resistance.

As the genetic backgrounds of the individuals in this experiment were not identical, it was not possible to distinguish whether the cost was due to a pleiotropic effect of the resistance gene or due to genes that are tightly linked. However, as the effect of reduced frequency of resistant individuals was observed across four populations it is highly likely that the penalty is due to a pleiotropic effect of the resistance mutation. For the purposes of using this information in models to predict the evolution of glyphosate resistance and to develop management strategies this distinction may not matter, providing the tightly linked genes are present in all glyphosate-resistant populations. All of the populations studied here have the translocation mechanism of resistance (Wakelin et al. 2004), but it affects resistance levels differently, as is shown by their different resistance levels (Table 1). If fitness is pleiotropic and resistance levels vary, it is possible that lower levels of glyphosate resistance might confer less of a fitness penalty than for a population with a high resistance level. However, as the populations here all had a similar decrease in frequency of resistance, the resistance level did not affect the level of fitness penalty.
ACKNOWLEDGMENTS
The authors gratefully acknowledge the ongoing support and financial contribution to this project by the GRDC (Project number UA 543). We also acknowledge technical assistance provided by Greg Naglis, Anthony Cathcart, and Ben Fleet.

REFERENCES


