Research Article

Received: 21 September 2012 Revised: 31 January 2013 Accepted article published: 19 February 2013 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/ps.3516

Cross-resistance to prosulfocarb and triallate in pyroxasulfone-resistant *Lolium rigidum*

Roberto Busi* and Stephen B Powles

Abstract

BACKGROUND: Plants can rapidly evolve resistance to herbicide in response to repeated selection. This study focuses on cross-resistance patterns observed in *Lolium rigidum* following pyroxasulfone recurrent selection.

RESULTS: The parental MR (multiresistant) population following four generations of pyroxasulfone recurrent selection evolved cross-resistance to prosulfocarb and triallate. At the recommended label rate of prosulfocarb or triallate (2000 g ha^{-1}), the progeny selected four times with pyroxasulfone (MR4) displayed 58 and 35% plant survival respectively. One additional cycle of prosulfocarb selection increased the resistance level to both prosulfocarb and triallate in the population MR4-P1. Prosulfocarb resistance is yet to be reported in *L. rigidum* field populations.

CONCLUSIONS: This study suggests that *L. rigidum* plants can rapidly evolve cross-resistance to several wheat-selective herbicides under recurrent selection of a single mode of action. Weed populations displaying broad-spectrum cross-resistance to several herbicide modes of action are increasing in frequency in intensive world agriculture. Proactive and integrated measures for resistance management need to be developed globally on appropriate herbicide use in crop rotations.

Supporting information may be found in the online version of this article.

Keywords: evolution; herbicide resistance; plant adaptation; population genetics; prosulfocarb; pyroxasulfone; triallate

1 INTRODUCTION

The evolution of herbicide-resistant weed populations is a significant issue in global agriculture.[1,2] High reliance on herbicides for weed control has resulted in herbicide resistance evolution in more than 200 weed species in many agroecosystems.[3] The response of plant populations to herbicide selection is determined by interactions between genotypes (i.e. biological and genetic factors) and the environment (i.e. intensity of herbicide selective pressure, herbicide mode of action, operational factors, plant stresses, etc.).[4] One of the most resistance-prone weed species is the genetically variable, obligate cross-pollinated annual diploid grass weed *Lolium rigidum* (Gaud.). *L. rigidum* in Australia is often present in high densities over vast areas, and herbicide resistance, since first reported,[5] now extends to many herbicides and is very widespread across Australian grain-growing regions.[6–9] A distinctive and adverse feature of resistance evolution in *L. rigidum*, at least in Australia, is that cross-resistance[10,11] and multiple resistance across some herbicide modes of action are common. The recent introductions of the pre-emergence herbicide prosulfocarb in 2008[12] and pyroxasulfone in 2012[13] have provided an effective tool for control of multiple-herbicide-resistant *L. rigidum* populations.[14] Prosulfocarb, triallate and pyroxasulfone are applied preplant and incorporated by crop seeding, are selective in wheat and induce similar symptoms in treated seeds preventing seedling emergence.[13,15,16] These herbicides are currently classified as distinct mode of action groups according to the HRAC classification (see www.plantprotection.org/HRAC), but similarities in selectivity spectrum and injury symptoms induced in grasses are well documented for thiocarbamates and acetamide herbicides [very-long-chain fatty acid (VLCFA) inhibitors].[17] Triallate and prosulfocarb (thiocarbamates, group N) inhibit fatty acid elongation and surface lipid (wax) biosynthesis, whereas pyroxasulfone (isoxazoline, VLCFA inhibitor, group K_3) has multiple enzymatic targets within the VLCFA synthesis pathway.[13]

Busi *et al*.[18] have recently shown that pyroxasulfone resistance evolution in *L. rigidum* can rapidly evolve under recurrent pyroxasulfone selection at below-label doses. In this study, an investigation was carried out to establish whether recurrent selection at below-label doses of pyroxasulfone can cause cross-resistance to the thiocarbamate herbicides prosulfocarb and triallate.

2 MATERIALS AND METHODS

2.1 Plant material

The *L. rigidum* population SLR31 (hereafter referred to as MR) has an extensive field history of herbicide selection[19] and exhibits multiple resistance to herbicides across different modes of

* *Correspondence to: Roberto Busi, Australian Herbicide Resistance Initiative, School of Plant Biology and Institute of Agriculture, University of Western Australia, 35 Stirling Highway, Crawley 6009, Western Australia. E-mail: roberto.busi@uwa.edu.au*

Australian Herbicide Resistance Initiative, School of Plant Biology and Institute of Agriculture, University of Western Australia, Crawley, Western, Australia

action.[18,20] This MR population is susceptible to the new herbicide pyroxasulfone,[14] but recurrent exposure to below-label doses of this herbicide has resulted in resistance evolution.[18] This pyroxasulfone-resistant progeny was selected by four consecutive generations with pyroxasulfone at 60 g ha^{-1} (MR1), 120 g ha^{-1} (MR2), 120 g ha^{-1} (MR3) and 240 g ha^{-1} (MR4) and will hereafter be referred to as MR4. As the pyroxasulfone-resistant progeny MR4 exhibited some degree of prosulfocarb resistance, those plants that survived a below-label dose of prosulfocarb (30 ± 5% survival at 1000 g ha^{-1}) were selected, and 13 survivors were allowed to cross-pollinate among themselves and were seed harvested. The seed obtained from these selected plants represented the selected progeny MR4-P1.

2.2 Pyroxasulfone, prosulfocarb and triallate cross-resistance

A preliminary experiment was conducted to assess the response to the recommended label rate of prosulfocarb, pyroxasulfone and triallate of the original parental MR population and each pyroxasulfone-selected or pyroxasulfone- and prosulfocarb-selected progeny. For each progeny there were at least 26 seeds tested with each herbicide at the recommended dose (Fig. 1).

The most resistant progenies were then compared with the original parental MR in final dose–response bioassays under identical environmental conditions. Plants were grown during the normal winter growing season in a natural outdoor environment. Seeds were germinated on 0.6% (w/v) agar medium and planted ($n = 26$ per pot) at the eruption of the primordial root at 0.5 cm depth in 2 L pots in commercial potting soil (50% peatmoss, 25% sand and 25% pine bark). After seed transplanting, the pots were treated with 0, 250, 500, 1000, 2000 and 4000 g prosulfocarb ha^{-1} or with 0, 12.5, 25, 50, 100, 200 and 400 g pyroxasulfone ha^{-1} or with 0, 250, 500, 1000, 2000 and 4000 g triallate ha^{-1}. There were two replications (pots) per herbicide dose, and each pot was the experimental unit. Wheat plants ($n = 15$ per pot) were also included, as wheat is known to be resistant to prosulfocarb, pyroxasulfone and triallate. A standard herbicide-susceptible *L. rigidum* population (VLR1) was used to verify the efficacy of each herbicide. *L. rigidum* plants were grown in optimal conditions and watered regularly (>80% field capacity). Nitrogen (as NH_4NO_3) was applied (50 mg kg^{-1}) at weekly intervals over the course of the experiment. After 21 days, emerged plants were counted to assess survival. These final dose–response studies were conducted twice.

2.3 Cross-resistance to other herbicide modes of action

An additional study was conducted to evaluate cross-resistance to several herbicide modes of action in the parental MR population and selected progenies in MR4 and MR4-P1 (supporting information Table S1). Selective herbicides known to be metabolised by wheat (chlorsulfuron, diclofop-methyl, *S*-metolachlor) versus herbicides toxic to and not metabolised by wheat (atrazine, glyphosate, paraquat, sethoxydim, sulfometuron, trifluralin) were tested at a discriminative dose (supporting information Table S1).[21–23]

2.4 Statistical analysis

Following appropriate statistical investigation, datasets of the two final dose–response studies were pooled prior to analysis. Survival data were subjected to non-linear regression analysis as described elsewhere.[18,24] The response to selection in the selected progenies was measured as the resistance index (RI) (the resistant/susceptible ratio) of estimated LD_{50} values. Statistical difference was assessed by the SI function in the *drc* package in the software program R v.2.14.1. Graphical data are presented as plant survival (%).

Table 1. Estimated LD_{50} values expressed as g pyroxasulfone, prosulfocarb or triallate ha^{-1} with standard errors in parentheses and resistance index (RI). Probability values (*P*) of difference between parental and selected populations in response to prosulfocarb were assessed by the SI function in the *drc* package in the software program R v.2.14.1. MR4 was selected 4 times with pyroxasulfone; MR4-P1 received one additional cycle of prosulfocarb selection

Population	LD_{50} (g ha^{-1})	RI[a]	*P*
Pyroxasulfone			
S	17 (1.6)	—	
MR	36 (3.2)	2.1	<0.01
MR4	231 (27)	13.6	<0.01
Wheat	369 (29)	21.7	<0.01
Prosulfocarb			
S	386 (118)	—	
MR	469 (92)	1.2	0.61
MR4	2828 (869)	7.3	<0.01
MR4-P1	>4000	>10.4	<0.01
Wheat	>4000	>10.4	<0.01
Triallate			
S	316 (35)	—	
MR	613 (80)	1.9	<0.01
MR4	1400 (173)	4.4	<0.01
MR4-P1	>2000	>6.3	<0.01
Wheat	1747 (193)	5.3	<0.01

[a] Resistance index is referred to the standard susceptible population S (VLR1).

3 RESULTS

3.1 Pyroxasulfone, prosulfocarb and triallate cross-resistance

The MR *L. rigidum* population is susceptible to pyroxasulfone, prosulfocarb and triallate (Fig. 1). However, as pyroxasulfone resistance progressively evolved by low-dose pyroxasulfone selection (see Busi *et al.*[18]), a concomitant shift towards resistance to prosulfocarb and triallate was observed. At each pyroxasulfone-selected generation there was greater plant survival following treatment with the recommended label dose of pyroxasulfone, prosulfocarb or triallate (Fig. 1). In the most resistant population (progeny MR4-P1) there was 77, 81 and 39% survival at the recommended label rate of pyroxasulfone, prosulfocarb and triallate, respectively.

Pyroxasulfone resistance was subsequently quantified in the MR4 population (selected four times with pyroxasulfone) by a dose–response experiment (Fig. 2A). Pyroxasulfone resistance in the progeny MR4 was 14-fold greater than in the susceptible standard population (Table 1). At the recommended field rate (100 g pyroxasulfone ha^{-1}), survival of MR4 plants was 80 ± 6%, compared with 10% observed in the original parental population MR and only 1% survival of susceptible standard plants (VLR1 population) (Fig. 2A). Wheat is known to be resistant to pyroxasulfone, and therefore, as expected, wheat plants displayed high pyroxasulfone resistance (Fig. 2A, Table 1). Pyroxasulfone resistance in MR4 was lower than in wheat plants ($P < 0.01$).

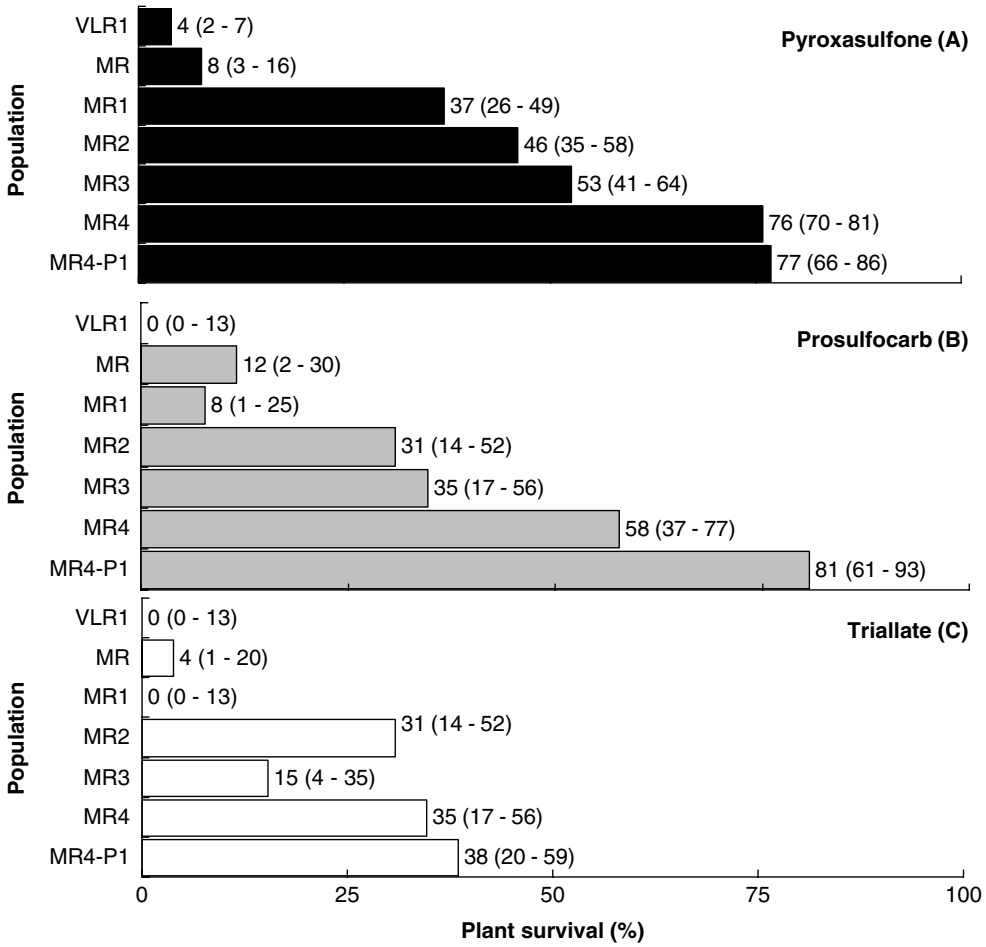

Figure 1. Plant survival (%) of the standard herbicide-susceptible *L. rigidum* population VLR1, the MR (multiresistant) parental population and each pyroxasulfone-selected progeny in response to the recommended dose of (A) pyroxasulfone (100 g ha^{-1}), (B) prosulfocarb (2000 g ha^{-1}) and (C) triallate (2000 g ha^{-1}). MR1, MR2, MR3 and MR4 were selected once, twice, 3 times and 4 times with pyroxasulfone respectively. MR4-P1 was additionally selected once with prosulfocarb. Mean values ($n \geq 26$) are indicated above each bar, and respective confidence intervals are given in parentheses.

The parental population MR was found to be as susceptible to prosulfocarb as the standard herbicide-susceptible population (VLR1) ($P > 0.82$) (Fig. 2B). However, the pyroxasulfone-resistant MR4 progeny exhibited cross-resistance to prosulfocarb. At the recommended prosulfocarb label rate (2000 g ha^{-1}) the progeny MR4 displayed 53% survival. Prosulfocarb resistance in MR4 was seven-fold, in comparison with the susceptible standard population (supporting information Table 1). Prosulfocarb resistance further increased under prosulfocarb selection as the progeny MR4-P1 displayed 77% plant survival at the recommended field rate (2000 g ha^{-1}). At the same prosulfocarb dose, the above-ground biomass was 52% of the untreated control. Prosulfocarb resistance exhibited by MR4-P1 plants was not significantly different from that observed in highly prosulfocarb-resistant wheat plants ($P > 0.21$) (Fig. 2B, Table 1).

The unselected parent MR was well controlled (only 5% survival) at 2000 g triallate ha^{-1} (Fig. 1C). A low level of triallate resistance was reported in an early study by Tardif and Powles[25] for this population. Here, similarly to that observed for prosulfocarb, triallate resistance evolution was evident (four-fold) in the progeny MR4. One further cycle of prosulfocarb selection further shifted the progeny MR4-P1 towards a greater triallate resistance level (more than six-fold) (Table 1). Progeny MR-P1 was as triallate resistant as the wheat crop ($P = 0.29$) (Fig. 2C, Table 1).

3.2 Cross-resistance to other herbicide modes of action

A single discriminative herbicide dose confirmed the population MR and selected progeny MR4 and MR4-P1 as multiple resistant to several herbicide modes of action, including chlorsulfuron (ALS), diclofop-methyl (ACCase), *S*-metolachlor (VLCFAs) and trifluralin (microtubule assembly), as previously shown.[19,20,25] A common feature of these herbicides is that they can all be metabolised by wheat. However, there was no resistance (supporting information Table S2) to herbicides that are toxic to and cannot be metabolised by wheat (sulfometuron, sethoxydim, glyphosate, paraquat).

4 DISCUSSION

4.1 Evolution of cross-resistance in *L. rigidum*

In a previous study the present authors showed that, under low-dose pyroxasulfone recurrent selection, *L. rigidum* plants progressively evolved resistance to pyroxasulfone.[18] Here, it is confirmed that recurrent selection with pyroxasulfone at below-label doses has caused the evolution of cross-resistance to prosulfocarb and triallate (Fig. 1). This is concerning, as these herbicides are all used to control *L. rigidum* and other grasses. It is notable that there was no cross-resistance to herbicides that cannot be metabolised by wheat, thus indicating that cross-resistance to pyroxasulfone, prosulfocarb and triallate is

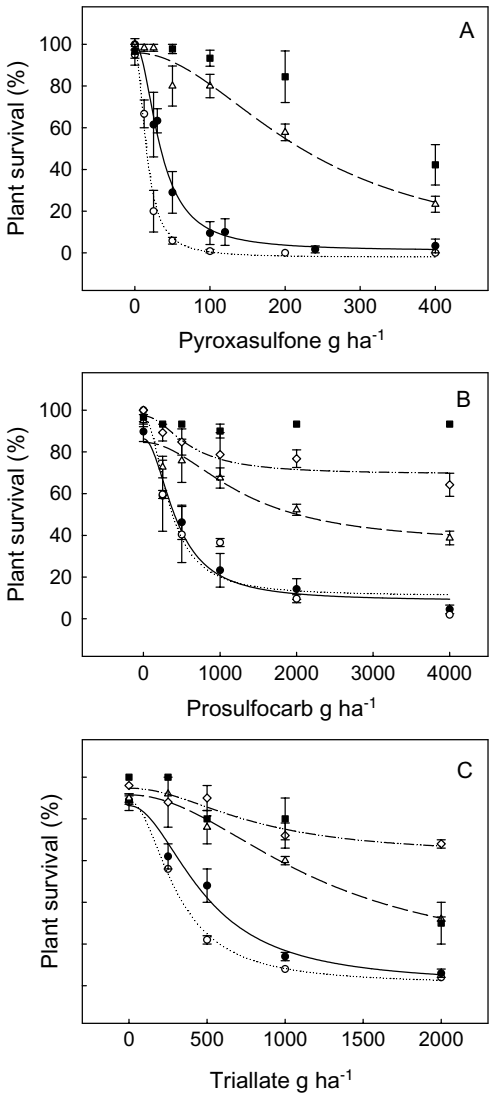

Figure 2. Pyroxasulfone (A), prosulfocarb (B) and triallate (C) dose–response study of parental MR *L. rigidum* population and progenies obtained by four generations of pyroxasulfone recurrent selection. Standard herbicide-susceptible population VLR1, dotted line and empty circles; parental MR, continuous line and solid circles; progeny MR4 selected 4 times with pyroxasulfone (once at 60 g ha^{-1}, twice at 120 g ha^{-1} and once at 240 g ha^{-1}), dashed line and empty circles; progeny MR4-P1, dashed-dotted line and empty diamonds. Wheat crop plants solid squares. Symbols are mean values of observed plant survival ± SE (n = 4). Lines represent the fitted log-logistic model.

probably metabolism based. The results reported here are also similar to those of earlier experiments in which recurrent low-dose diclofop-methyl selection resulted in a clear shift towards diclofop-methyl resistance and cross-resistance to chemically dissimilar, wheat-metabolised herbicides.[26–28] The parental *L. rigidum* MR population was originally selected by 20 years of repeated herbicide field use, which was beneficial for genetic trait(s) effective against several herbicide modes of action, mainly through a higher capacity for herbicide metabolism, probably due to cytochrome P450 enzymes.[19,21,25] In the present study it has been demonstrated that, over three generations, the use of a metabolisable herbicide (pyroxasulfone) at a low dose enabling a number of plants to survive (i.e. a minimum of ten selected individuals at each generation) resulted in cross-resistance to several herbicides (see also Busi *et al*.[18]). In this *L. rigidum* population, high-level cross-resistance was evident to all the wheat-selective herbicides tested.

4.2 Mechanistic basis of cross-resistance

Metabolic herbicide detoxification is a key trait providing the basis for both cross-resistance in *L. rigidum* and selectivity in wheat plants.[29] In *L. rigidum*, cross-resistance to several herbicide modes of action is often endowed by herbicide metabolism via enhanced rates of cytochrome P450 enzymatic activity.[30] The *L. rigidum* population used in this study has shown an enhanced capacity for herbicide metabolism of sulfonylureas mediated by cytochrome P450.[21,31] However, in this population, P450-mediated metabolism of the herbicide triallate was not evident,[25] whereas there is some evidence of GST-mediated pyroxasulfone resistance (Busi R *et al*., unpublished). In other plant species it appears that thiocarbamate and acetamide herbicides require the involvement of P450 and/or GST enzymes.[17,32,33] Future research is warranted to define the biochemical basis of resistance and test whether more than one mechanism is required to endow cross-resistance to pyroxasulfone, prosulfocarb and triallate.

4.3 Are evolutionary studies leading to successful resistance management?

From 2012, three chemically different pre-emergent herbicide modes of action, pyroxasulfone (VLCFA inhibitor), prosulfocarb and triallate (lipid synthesis inhibitors) and trifluralin (microtubule formation inhibitor) can be used in rotation for *L. rigidum* control in wheat in Australia. It is emphasised that pyroxasulfone is a novel pre-emergence herbicide for *L. rigidum* control that has been commercialised from 2012 in Australia, United States/Canada and some other countries. Thus, information regarding the evolution of cross-resistance to new herbicides is extremely valuable for farmers and agronomists. Rotation between or mixtures of pyroxasulfone, prosulfocarb, triallate and trifluralin is recommended as part of an integrated programme of *L. rigidum* management. Preliminary modelling simulations have shown that rotation between prosulfocarb, pyroxasulfone and trifluralin is probably an effective measure to delay the evolution of resistance in *L. rigidum* populations (Renton M *et al*., unpublished). However, this study has shown that cross-resistance across these herbicides can rapidly evolve in *L. rigidum*. This capacity of *L. rigidum* plants to evolve broad-spectrum resistance, as demonstrated in this and several other studies focusing on *L. rigidum*,[18,20,26] is a major agronomic and commercial concern. Resistance mechanisms (genetic traits) can be easily accumulated in *L. rigidum* individuals owing to gene flow through obligate cross-pollination.[34] Grass weed species such as *L. rigidum*, *Alopecurus myosuroides* or *Echinochloa phyllopogon* possess a large array of genetic traits, which can limit herbicide damage and contribute to herbicide resistance in plants under selection.[26,35,36] This could explain cases of resistance to similar herbicide modes of action documented in *Alopecurus myosuroides*, *Avena fatua*, *Echinochloa* spp. and *Lolium* spp.[12,19,37–39] Thus, herbicide resistance management based on the use of novel herbicides to control already resistant weed populations is useful but not a panacea. Proactive and integrated measures that embrace diversity for the management of multiresistant *L. rigidum* need to be instituted. For example, in spite of the high frequency of multiresistant *L. rigidum* populations infesting crop fields, Australian growers have successfully managed herbicide resistant *L. rigidum*[40] by implementing herbicide (e.g. double

knock)[41] and/or non-chemical strategies to control herbicide-resistant weeds (e.g. weed seed control at harvest).[42,43] This recurrent selection study has made it possible to correlate the effects of repeated herbicide selection and the potential for evolved cross-resistance in the target weed *L. rigidum*. A greater evolutionary focus in herbicide resistance research could lead to a greater overall understanding of the dynamics and factors involved in resistance and cross-resistance evolution.[44] Thus, the authors believe that recurrent selection studies can raise awareness of the rapid evolution of resistance in weeds and thus help to design effective strategies for proactive resistance management. The adoption of integrated weed management strategies is equally vital to sustaining herbicide longevity in global agriculture.

ACKNOWLEDGEMENTS

The Australian Herbicide Resistance Initiative (AHRI) receives funding from the Grains Research and Development Corporation of Australia (GRDC). This research has been partially funded by the Rural Industries Research and Development Corporation (RIRDC) under the National Weeds and Productivity Programme. Special thanks to Mr Andrea Pettigiani for skilled research assistance.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Beckie HJ and Tardif FJ, Herbicide cross resistance in weeds. *Crop Prot* **35**:15–28 (2012).
2 Powles SB and Yu Q, Evolution in action: plants resistant to herbicides. *Annu Rev Plant Biol* **61**:317–347 (2010).
3 Heap IM, *International Survey of Herbicide Resistant Weeds*. [Online]. Available: http://www.weedscience.org [accessed 27 June 2012].
4 Jasieniuk M, Brûlé-Babel AL and Morrison IN, The evolution and genetics of herbicide resistance in weeds. *Weed Sci* **44**(1):176–193 (1996).
5 Heap J and Knight R, A population of ryegrass tolerant to the herbicide diclofop-methyl. *J Aust Inst Agric Sci* **48**:156–157 (1982).
6 Owen MJ and Powles SB, Multiple herbicide resistant *Lolium rigidum* (annual ryegrass) now dominates across the Western Australian grain belt. *Weed Res* **under review** (2013).
7 Owen MC, Walsh MJ, Llewellyn RS and Powles SB, Widespread evolution of multiple herbicide resistance in annual ryegrass (*Lolium rigidum*) populations within the Western Australian wheat belt. *Aust J Agric Res* **58**(7):711–718 (2007).
8 Broster JC and Pratley JE, A decade of monitoring herbicide resistance in *Lolium rigidum* in Australia. *Aust J Exp Agric* **46**(9):1151–1160 (2006).
9 Boutsalis P, Gill G and Preston C, Incidence of herbicide resistance in rigid ryegrass (*Lolium rigidum*) across southeastern Australia. *Weed Technol* **26**(3):391–398 (2012).
10 Hall LM, Holtum JAM and Powles SB, Mechanisms responsible for cross resistance and multiple resistance, in *Herbicide Resistance in Plants: Biology and Biochemistry*, ed. by Powles SB and Holtum JAM. Lewis Publisher, Boca Raton, FL, pp. 243–261 (1994).
11 Heap IM and Knight R, The occurrence of herbicide cross resistance in a population of annual ryegrass, *Lolium rigidum*, resistant to diclofop-methyl. *Aust J Agric Res* **37**:149–156 (1986).
12 Bailly GC, Dale RP, Archer SA, Wright DJ and Kaundun SS, Role of residual herbicides for the management of multiple herbicide resistance to ACCase and ALS inhibitors in a black-grass population. *Crop Prot* **34**:96–103 (2012).
13 Tanetani Y, Kaku K, Kawai K, Fujioka T and Shimizu T, Action mechanism of a novel herbicide, pyroxasulfone. *Pestic Biochem Physiol* **95**(1):47–55 (2009).
14 Walsh MJ, Fowler TM, Crowe B, Ambe T and Powles SB, The potential for pyroxasulfone to selectively control resistant and susceptible rigid ryegrass (*Lolium rigidum*) biotypes in Australian grain crop production systems. *Weed Technol* **25**(1):30–37 (2011).
15 Fuerst EP, Understanding the mode of action of the chloroacetamide and thiocarbamate herbicides. *Weed Technol* **1**(4):270–277 (1987).
16 Baldwin A, Francis D, Rogers HJ and Harwood JL, The inhibition of fatty acid elongation by pebulate can be effectively counteracted by the safener dichlormid. *Biochem Soc Trans* **28**:650–651 (2000).
17 Gronwald JW, Lipid biosynthesis inhibitors. *Weed Sci* **39**(3):435–449 (1991).
18 Busi R, Gaines TA, Walsh MJ and Powles SB, Understanding the potential for resistance evolution to the new herbicide pyroxasulfone: field selection at high doses versus recurrent selection at low doses. *Weed Res* **52**(6):489–499 (2012).
19 Burnet MWM, Barr AR and Powles SB, Chloroacetamide resistance in rigid ryegrass (*Lolium rigidum*). *Weed Sci* **42**:153–157 (1994).
20 Busi R, Vila-Aiub MM and Powles SB, Genetic control of cytochrome P450-based enhanced herbicide metabolism in a *Lolium rigidum* population. *Heredity* **106**:817–824 (2011).
21 Christopher JT, Powles SB, Liljegren DR and Holtum JAM, Cross-resistance to herbicides in annual ryegrass (*Lolium rigidum*) II. Chlorsulfuron resistance involves a wheat-like detoxification system. *Plant Physiol* **95**(4):1036–1043 (1991).
22 Tardif FJ, Holtum JAM and Powles SB, Occurrence of a herbicide-resistant acetyl-coenzyme-A carboxylase mutant in annual ryegrass (*Lolium rigidum*) selected by sethoxydim. *Planta* **190**(2):176–181 (1993).
23 Shimabukuro RH, Walsh WC and Hoerauf RA, Metabolism and selectivity of diclofop-methyl in wild oat and wheat. *J Agric Food Chem* **27**(3):615–623 (1979).
24 Knezevic I, Streibig JC and Ritz C, Utilizing R software package for dose–response studies: the concept and data analysis. *Weed Technol* **21**:840–848 (2007).
25 Tardif FJ and Powles SB, Effect of malathion on resistance to soil-applied herbicides in a population of rigid ryegrass (*Lolium rigidum*). *Weed Sci* **47**(3):258–261 (1999).
26 Busi R, Neve P and Powles SB, Evolved polygenic herbicide resistance in *Lolium rigidum* by low-dose herbicide selection within standing genetic variation. *Evolutionary Applic* **6**(2):231–242 (2012).
27 Neve P and Powles SB, Recurrent selection with reduced herbicide rates results in the rapid evolution of herbicide resistance in *Lolium rigidum*. *Theor Appl Genet* **110**(6):1154–1166 (2005).
28 Manalil SV, Busi R, Renton M and Powles SB, Rapid evolution of herbicide resistance by low herbicide dosages. *Weed Sci* **59**(1):210–217 (2011).
29 Christopher JT, Powles SB, Liljegren DR and Holtum JAM, Cross resistance to herbicides in annual ryegrass (*Lolium rigidum*). II. Chlorsulfuron resistance involves a wheat-like detoxification system. *Plant Physiol* **95**(4):1036–1043 (1991).
30 Preston C, Tardif FJ, Christopher JT and Powles SB, Multiple resistance to dissimilar herbicide chemistries in a biotype of *Lolium rigidum* due to enhanced activity of several herbicide degrading enzymes. *Pestic Biochem Physiol* **54**(2):123–134 (1996).
31 Christopher JT, Powles SB and Holtum JAM, Resistance to acetolactate synthase-inhibiting herbicides in annual ryegrass (*Lolium rigidum*) involves at least two mechanisms. *Plant Physiol* **100**(4):1909–1913 (1992).
32 Baldwin A, Rogers HJ, Francis D and Harwood JL, Fatty acid elongation is important in the activity of thiocarbamate herbicides and in safening by dichlormid. *J Exp Bot* **54**(385):1289–1294 (2003).
33 Han S and Hatzios KK, Uptake, translocation, and metabolism of [^{14}C]pretilachlor in fenclorim-safened and unsafened rice seedlings. *Pestic Biochem Physiol* **39**(3):281–290 (1991).
34 Busi R, Yu Q, Barrett-Lennard R and Powles S, Long distance pollen-mediated flow of herbicide resistance genes in *Lolium rigidum*. *Theor Appl Genet* **117**(8):1281–1290 (2008).
35 Petit C, Duhieu B, Boucansaud K and Délye C, Complex genetic control of non-target-site-based resistance to herbicides inhibiting acetyl-coenzyme A carboxylase and acetolactate-synthase in *Alopecurus myosuroides* Huds. *Plant Sci* **178**(7):501–509 (2010).
36 Yasuor H, Milan M, Eckert JW and Fischer AJ, Quinclorac resistance: a concerted hormonal and enzymatic effort in *Echinochloa phyllopogon*. *Pest Manag Sci* **68**(1):108–115 (2012).

37 Fischer AJ, Ateh CM, Bayer DE and Hill JE, Herbicide-resistant *Echinochloa oryzoides* and *E. phyllopogon* in California *Oryza sativa* fields. *Weed Sci* **48**(2):225–230 (2000).
38 Beckie HJ, Warwick SI and Sauder CA, Basis for herbicide resistance in Canadian populations of wild oat (*Avena fatua*). *Weed Sci* **60**(1):10–18 (2011).
39 Kern AJ, Myers TM, Jasieniuk M, Murray BG, Maxwell BD and Dyer WE, Two recessive gene inheritance for triallate resistance in *Avena fatua* L. *J Heredity* **93**(1):48–50 (2002).
40 Llewellyn RS, D'Emden FH, Owen MJ and Powles SB, Herbicide resistance in rigid ryegrass (*Lolium rigidum*) has not led to higher weed densities in Western Australian cropping fields. *Weed Sci* **57**(1):61–65 (2009).
41 Borger CP and Hashem A, Evaluating the double knockdown technique: sequence, application interval, and annual ryegrass growth stage. *Aust J Agric Res* **58**:265–271 (2007).
42 Walsh MJ and Powles SB, Management strategies for herbicide-resistant weed populations in Australian dryland crop production systems. *Weed Technol* **21**(2):332–338 (2007).
43 Walsh MJ, Harrington RB and Powles SB, Harrington seed destructor: a new nonchemical weed control tool for global grain crops. *Crop Sci* **52**(3):1343–1347 (2012).
44 Neve P, Vila-Aiub MM and Roux F, Evolutionary-thinking in agricultural weed management. *New Phytol* **184**(4):783–793 (2009).

