Research Article

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Non-target-site-based resistance to ALS-inhibiting herbicides in six *Bromus rigidus* populations from Western Australian cropping fields

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Abstract

BACKGROUND: *Bromus rigidus* is a common weed species that has increased in cropping fields owing to limited control options. During a random field survey in Western Australia, six *B. rigidus* populations that had survived in-crop weed control programmes were collected. The study aimed to determine the resistance profile of these six populations.

RESULTS: Based on dose–response studies, all six *B. rigidus* populations had a low-level resistance to sulfosulfuron and sulfometuron (both sulfonylurea herbicides) while remaining susceptible to herbicides with other modes of action. ALS *in vitro* activity assays revealed no differences in enzyme sensitivity between susceptible and resistant populations, while the use of malathion (a cytochrome P450 inhibitor) in combination with sulfosulfuron caused the resistant populations to behave like the susceptible population.

CONCLUSION: This study established that these six *B. rigidus* populations have a low-level resistance to the ALS-inhibiting sulfonylurea herbicides, but are able to be controlled by other herbicide modes of action. The low-level, malathion-reversible resistance, together with a sensitive ALS, strongly suggest that a non-target-site enhanced metabolism is the mechanism of resistance.

Keywords: resistance survey; resistance evolution; *Bromus* spp.; ALS resistance

1 INTRODUCTION

Rigid brome (*Bromus rigidus* Roth) is a self-pollinated winter annual grass of Mediterranean origin. *Bromus* species are commonly found on lighter sandy soil across the wheat belt of southern Australia and are well adapted to the hot dry summers and mild wet winters of this climate.[1–4] In recent years, *Bromus* spp. have become more abundant in cereal-growing regions,[5] largely owing to increased cereal cropping frequency and the widespread adoption of conservation tillage. This technique often relies heavily on herbicide use, with limited in-crop herbicide options for *Bromus* control.[2,4,6] *Bromus* spp. are competitive in both crops and pastures, resulting in reduced crop growth and yield;[5] furthermore, its seeds can contaminate grain.[7] On account of seed dormancy, germination of *Bromus* spp. can extend over the crop-growing season,[4,8,9] with cohorts emerging in crop that require selective herbicide treatments.

Many herbicides target the plastidic enzyme acetolactate synthase (ALS), and these ALS herbicides are used to control a wide range of weed species in a variety of field crops. The acetyl-coenzyme A carboxylase (ACCase)- and ALS-inhibiting herbicides are the most important for *Bromus* control in Australia, and the newer ALS-inhibiting herbicides sulfosulfuron (Monza®) and mesosulfuron-methyl (Atlantis®) provide selective control of *Bromus* spp. in wheat.[10] However, these soil-residual herbicides can have persistence issues[11] and must be applied no later than the three-leaf growth stage to achieve good control. The continued use of ALS herbicides has led to the widespread evolution of ALS-herbicide-resistant weed populations (reviewed in Saari *et al.*,[12] Tranel and Wright[13] and Powles and Yu[14]). Worldwide, there are now 113 species with resistance to the ALS herbicides.[15] While to date there are no published cases of ALS-herbicide-resistant *Bromus* populations in Australia, ACCase herbicide resistance in *Bromus* species is known.[15,16] *Bromus tectorum* (downy brome), a widespread and competitive weed in crops in the Pacific Northwest of the United States, has evolved sulfonylurea (SU)-class ALS herbicide resistance.[15,17,18]

A recent survey in Western Australia found evidence of *B. rigidus* biotypes that were difficult to control during the cropping phase.[19] The focus of the present study was to assess resistance to ALS-inhibiting SU herbicides in *B. rigidus* populations from Australia. The study characterises six *B. rigidus* populations resistant to ALS herbicides and demonstrates that resistance is likely, and

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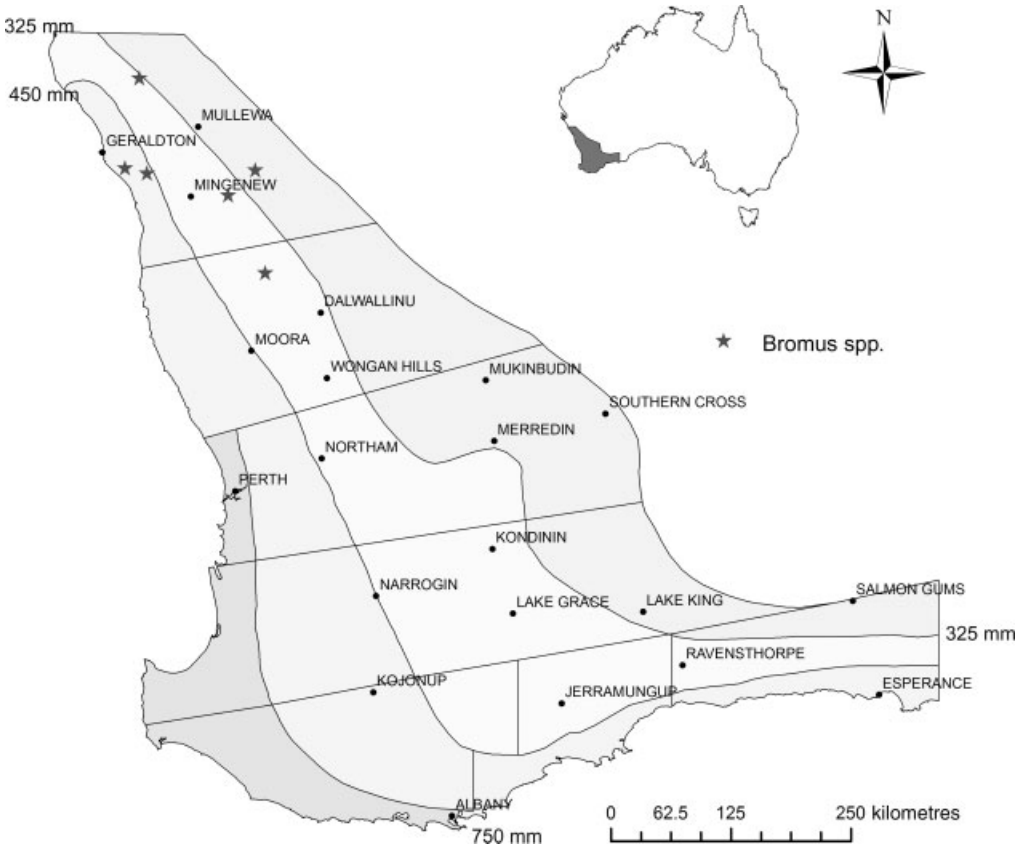

Figure 1. Map of south-western Western Australia, showing the agronomic zones of the grain belt where *B. rigidus* samples were collected for herbicide resistance testing. Average annual rainfall isohyets are shown.

unexpectedly, due to non-target-site-based enhanced rates of herbicide metabolism.

2 MATERIALS AND METHODS

2.1 Seed collection and germination

In 2005, 677 cropping fields were randomly visited across the Western Australian grain belt (Fig. 1) (see Owen and Powles[19] for detailed methodology). A small number of fields were found to have high numbers of *B. rigidus* plants, and seeds from six field populations were collected for resistance screening. All populations tested came from the northern agricultural region (Fig. 1), as plants in the southern region had immature seed at the time of collection.

B. rigidus seeds (after-ripened and thus non-dormant) were germinated on water solidified with agar (1%) in 500 mL plastic containers for 7–10 days under ambient conditions in the laboratory. For each population and herbicide treatment, 50 seedlings were transplanted into plastic trays containing a standard potting mix (50% composted pine bark, 25% peat and 25% river sand). Seedlings were grown at the University of Western Australia in an outdoor plot during the normal growing season (May to September) and were watered and fertilised regularly.

2.2 Single-rate herbicide resistance testing

A range of herbicides inhibiting ACCase, ALS, photosystem I or 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) were tested at recommended field rates (Table 1) to determine the susceptibility of the *B. rigidus* biotypes to several different herbicide chemistries. When seedlings reached the 2–3-leaf stage, they were treated with herbicide (see Owen and Powles[19] for details of the spraying equipment used). Twenty-one days later, survival of plants was assessed by inspecting the growing points: if these were chlorotic or necrotic and there were no new tillers forming, and leaf tissue was easily fragmented, the plants were considered to be dead. There were 50 plants per herbicide treatment, with two replicates for each biotype. A previously verified susceptible population (from South Australia) was used as a control for each herbicide treatment. For all herbicides, 100% mortality occurred in the known susceptible population (data not shown).

2.3 Dose response to ALS-inhibiting herbicides

Seedlings from each of the putative resistant populations were tested during May–September 2009 to quantify the level of resistance to ALS herbicides. Plants (two-leaf stage) were sprayed with sulfometuron 750 g kg^{-1} WG together with 0.25% (v/v) wetting agent BS1000 at 0, 3.75, 7.5, 15, 30, 60, 120 and 240 g ha^{-1}, with imazamox 700 g kg^{-1} WG together with 2% crop oil (Hasten; Victorian Chemicals Australia) at 0, 8, 16, 32 and 64 g ha^{-1} or with sulfosulfuron 750 g kg^{-1} WG at 0, 37.5, 75, 150, 300 and 600 g ha^{-1}. Assessment of survival was conducted 28 days after spraying, and the above-ground tissue was harvested, dried at 70 °C for 72 h and weighed. The experiment, performed twice during the growing season, was randomised with 20 seedlings per pot, and had three replicates per treatment.

Table 1. Resistance status across differing herbicide chemistries for all six resistant biotypes of *B. rigidus*. S denotes that the populations were completely susceptible (i.e. all plants died) to the herbicide, and R denotes that the populations were resistant (>95% of plants survived)

Herbicide chemical class	Active ingredient	Herbicide mode of action	Rate (g ha^{-1})	Resistance status
Aryloxyphenoxypropionate	Fluazifop	Inhibition of ACCase	78	S
	Haloxyfop	Inhibition of ACCase	104	S
Cyclohexanedione	Sethoxydim	Inhibition of ACCase	186	S
	Clethodim	Inhibition of ACCase	60	S
Sulfonylurea	Sulfometuron	Inhibition of ALS	15	R
	Sulfosulfuron	Inhibition of ALS	37.5	R
Imidazolinone	Imazapic + imazapyr	Inhibition of ALS	7 + 21	S
	Imazamox	Inhibition of ALS	31.5	S
Bipyridyl	Paraquat	Inhibition of photosystem I	300	S
Glycine	Glyphosate	Inhibition of EPSPS	540	S

2.4 Effect of malathion on sulfosulfuron resistance

Malathion is an organophosphate insecticide known to be an inhibitor of cytochrome P450 monooxygenases. Malathion is a synergist for the herbicide chlorsulfuron in *Lolium rigidum*[20,21] and the herbicide primisulfuron in *Zea mays* (maize).[22] Preliminary studies showed that there was no adverse effect on the seedling growth of *B. rigidus* when malathion was applied alone at 1000 g ha^{-1}; therefore, this rate was used in this study. During May–July 2011, seeds of each of the resistant *B. rigidus* populations were sprayed with sulfosulfuron 750 g kg^{-1} WG at 0, 18.75, 37.5, 75, 150, 300 and 600 g ha^{-1}, with or without malathion. Malathion was applied 30 min prior to the application of sulfosulfuron using the herbicide sprayer. Plants were assessed 21 days after treatment, and the above-ground tissue was harvested, dried and weighed as above. The experiment was randomised, with 20 seedlings per pot, with or without malathion, with two replicates per treatment. A second susceptible population collected from the south-west of Western Australia was also used as a control in these experiments.

2.5 Statistical analyses for herbicide treatment studies

Non-linear regression was used to estimate the herbicide rate causing 50% mortality (LD_{50}) or growth reduction (GR_{50}) of plants using Sigma Plot software (v.11.0). The R : S (resistant : susceptible) ratio of estimated LD_{50} values was used as the measure of resistance. A *t*-test ($P = 0.05$) was used to determine the level of significance. Mortality dose–response graphs are presented with untransformed data. The data were fitted to the log-logistic model[23]

$$y = C + \left[\frac{D - C}{1 + (X/\mathrm{ED}_{50})^b}\right]$$

where C is the lower limit, D is the upper limit, b is the slope and ED_{50} is the dose causing 50% reduction. When it was not possible to fit a log-logistic model to the biomass data, an exponential decay model was used. Datasets were analysed by ANOVA, and LSD ($P = 0.05$) was used to determine significant differences between populations.

2.6 *In vitro* ALS enzyme activity

ALS enzyme activity in leaf blades (1 g of young fully expanded tissue collected from 4–8 individuals for each replicate) was measured colorimetrically using a method modified from Ray.[24] Leaf blades were snap frozen and pulverised in liquid nitrogen, then extracted in 3 mL of cold grinding buffer [0.1 M K_2HPO_4, pH 7.5, 1 mM sodium pyruvate, 0.5 mM $MgCl_2$, 0.5 mM thiamine pyrophosphate (TPP), 10 µM flavine adenine dinucleotide (FAD), 1% (w/v) polyvinylpyrrolidone, 10% (v/v) glycerol, 1 mM dithiothreitol (DTT), 1 mM phenylmethylsulphonyl fluoride] and clarified by centrifugation at 12 000 × *g* at 4 °C. Using PD-10 (Pharmacia) columns, the supernatant was desalted into phosphate buffer (0.1 M K_2HPO_4, pH 7.5, 20 mM sodium pyruvate, 0.5 mM $MgCl_2$, 1 mM DTT) and then assayed for ALS activity in a 250 µL reaction containing 185 µL of desalted extract.[24] The sensitivity of ALS to inhibitors was assessed by adding technical-grade sulfometuron (Nufarm) at 0, 0.0001, 0.001, 0.01, 0.1, 1, 10 or 100 µM to the reaction. Reactions were incubated at 30 °C for 60 min (during which time the reaction rate was linear) and terminated with the addition of H_2SO_4 to 0.55 N (H_2SO_4 was added to negative controls at 0 min). Production of acetoin was monitored at 530 nm following treatment of the reaction mixture with creatine and α-naphthol, with 0–75 nmol pure acetoin being used as a standard. The protein concentration in the leaf extracts was measured as in Bradford,[25] using 0–25 µg of bovine serum albumin as a standard and colour development with BioRad dye reagent concentrate. Each enzyme assay was performed with four independent biological replicates, and protein concentration was measured in duplicate in each sample.

3 RESULTS

3.1 Single-rate herbicide resistance testing

Six *B. rigidus* populations were screened with a range of herbicides with known activity on this species. All six populations responded similarly, being resistant to the ALS-inhibiting SU-class herbicides sulfometuron and sulfosulfuron but susceptible to the imidazolinone (IMI)-class ALS herbicides and the inhibitors of ACCase, EPSPS and photosystem I (Table 1).

3.2 Dose response to ALS-inhibiting herbicides

Detailed dose–response studies confirmed that all six *B. rigidus* populations tested were resistant to the ALS-inhibiting herbicides sulfometuron and sulfosulfuron (Tables 2 and 3). The susceptible (S) biotype was controlled at field rates, in contrast to the six resistant (R) biotypes (Figs 2 and 3a). The lethal dose of SU herbicides required to kill 50% of the R biotypes was 3–4 times greater than that required to kill the S biotype for sulfometuron (Table 2), and 6–11 times greater for sulfosulfuron (Table 3). However, biomass data revealed that the growth of the R populations was clearly affected by sulfosulfuron, with high herbicide rates significantly reducing the biomass (Fig 3b).

Table 2. LD_{50} values (standard errors in parentheses; $n = 3$) of *B. rigidus* populations treated with sulfometuron. R/S ratios were calculated as the ratio of LD_{50} values of resistant and susceptible populations[a]

	Sulfometuron	
Biotype	LD_{50} (g ha^{-1})	R/S ratio of LD_{50}
Susceptible	10 (0.8)	n/a
R1	30 (2.4)	3
R2	33 (3.7)	3.3
R3	31 (2.2)	3.1
R4	30 (2.9)	3
R5	38 (1.1)	3.8
R6	41 (2.0)	4.1

[a] LD_{50}: the dose lethal to 50% of the population; R: resistant; S: susceptible.

Table 3. LD_{50} values (standard errors in parentheses; $n = 2$) of *B. rigidus* populations treated with sulfosulfuron ± 1000 g ha^{-1} malathion. R/S ratios were calculated as the ratio of LD_{50} values of resistant and susceptible populations

	Sulfosulfuron (no malathion)		Sulfosulfuron (plus malathion)	
Biotype	LD_{50} (g ha^{-1})	R/S ratio of LD_{50}	LD_{50} (g ha^{-1})	R/S ratio of LD_{50}
Susceptible	21 (1.3)	n/a	6 (0.1)	n/a
R1	183 (21.0)	8.7	16 (0.38)	2.6
R2	166 (18.6)	7.9	10 (0.01)	1.6
R3	173 (11.6)	8.2	16 (0.02)	2.6
R4	239 (27.7)	11.3	13 (0.26)	2.1
R5	140 (12.9)	6.6	12 (0.01)	2.0
R6	127 (8.2)	6.0	11 (0.16)	1.8

3.3 Effect of malathion on sulfosulfuron resistance

When malathion was applied alone at 1000 g ha^{-1}, there was no effect on survival (Fig. 3a) or biomass (data not shown) of either the S or R biotypes. In the presence of malathion, the R biotypes became susceptible to sulfosulfuron and the mortality of the already susceptible biotypes was increased further (Fig. 3a, Table 3). Similar results were obtained for the above-ground biomass (data not shown).

3.4 *In vitro* ALS activity and inhibition

All R biotypes showed similar ALS activity, and therefore one population was selected as a representative sample for testing of the sensitivity of the enzyme to technical-grade sulfometuron. In the absence of the herbicide, ALS activity was the same in leaf extracts from both the R and S biotypes ($P = 0.16$), with an overall average activity of 1.3 ± 0.1 µmol acetoin formed min^{-1} mg^{-1} protein. Sulfometuron, when added to the reactions, almost completely inhibited ALS activity from both the S and R biotypes at concentrations of 0.1 µM and above (Fig. 4). The I_{50} for the S biotype was 0.00314 ± 0.0017 µM and for the R biotype 0.0019 ± 0.0008 µM, which were not significantly different ($P = 0.878$). Therefore, the whole-plant resistance evident in the R biotypes is not due to target-site ALS enzyme insensitivity.

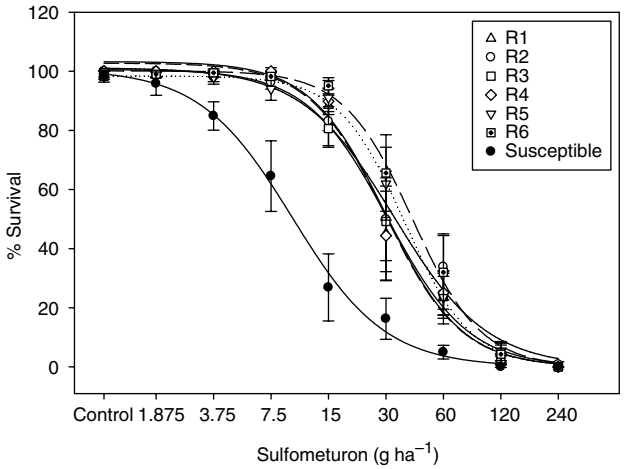

Figure 2. Dose–response curves for survival of an ALS-susceptible *B. rigidus* population and the six ALS-resistant *B. rigidus* populations treated with a range of sulfometuron doses. Each data point represents the mean percentage survival ± SE of three replicate treatments.

4 DISCUSSION

This study is the first report to characterise ALS herbicide resistance in Australian populations of *B. rigidus*. Six biotypes were found to have relatively low-level resistance to the SU herbicides sulfosulfuron and sulfometuron (Table 2, Fig. 2). Similarly, a recent survey in Spain found that the level of resistance to sulfosulfuron in *B. diandrus* populations randomly collected from wheat fields could generally be classified only as intermediate (15–85% reduction in biomass at field rates of sulfosulfuron) rather than high (≤15% biomass reduction).[26] Resistance in the Australian populations characterised in the present study was specific to the SU group of ALS-inhibiting herbicides, as the R biotypes were susceptible to the IMI herbicides as well as to herbicides with different modes of action (Table 1).

Resistance to ALS-inhibiting herbicides can be target site based, commonly owing to one of several amino acid substitutions at the herbicide-binding site of the ALS enzyme, resulting in reduced sensitivity to the herbicide. In other cases, non-target-site-based resistance can be the result of herbicide detoxification via enhanced rates of metabolism of the herbicide molecule (reviewed in Powles and Yu[14]). Enhanced metabolism catalysed by cytochrome P450 monooxygenases is a prominent resistance mechanism found across six different herbicide groups, which has evolved in at least nine weed species including *Bromus* species.[14] This mechanism of resistance has been observed in several populations of *L. rigidum* from Australia and *Alopecurus myosuroides* from Europe, where these biotypes have displayed non-target-site cross-resistance across several herbicide modes of action, including herbicide groups never used (reviewed in Tranel and Wright[13] and Powles and Yu[14]).

Synergistic interactions between organophosphate insecticides, which inhibit cytochrome P450 activity, and SU herbicides have been well documented,[22,27] and previous studies have shown that malathion is an effective inhibitor of P450-mediated herbicide resistance in weed species such as *L. rigidum*.[20,28] In the present study there are three lines of evidence suggesting that SU resistance in these *B. rigidus* populations is due to P450-mediated enhanced metabolism of the herbicides rather than a target-site mutation: (1) the relatively low-level resistance to sulfosulfuron and sulfometuron, further illustrated by the decrease in biomass

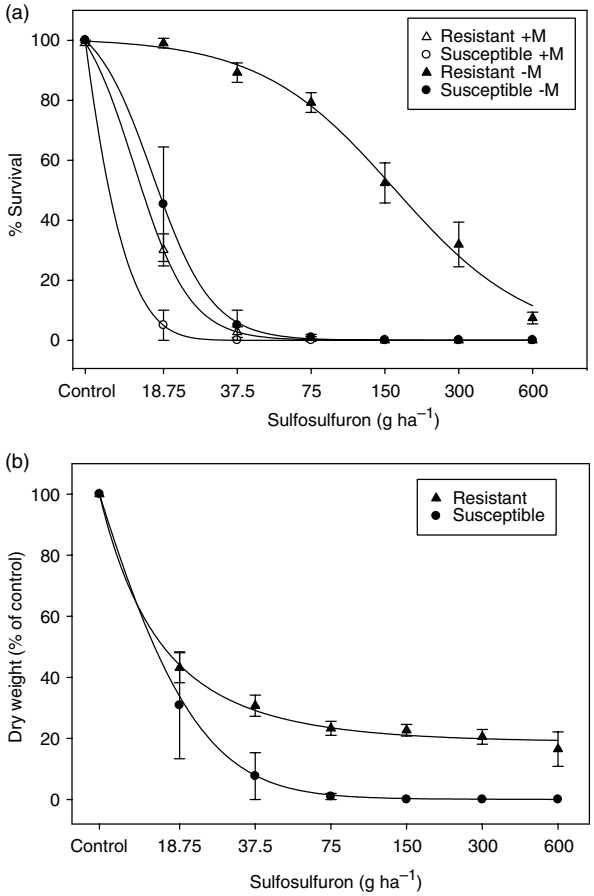

Figure 3. Dose–response curves for (a) survival of an ALS-susceptible *B. rigidus* population and the ALS-resistant *B. rigidus* populations treated with a range of sulfosulfuron doses plus or minus 1000 g ha^{-1} malathion (M) and (b) plant biomass of an ALS-susceptible *B. rigidus* population and the ALS-resistant *B. rigidus* populations treated with a range of sulfosulfuron doses. Each data point represents the mean percentage survival ± SE of two replicate treatments. As the six resistant populations all gave a similar response, data have been averaged across these populations and presented as one curve for clarity.

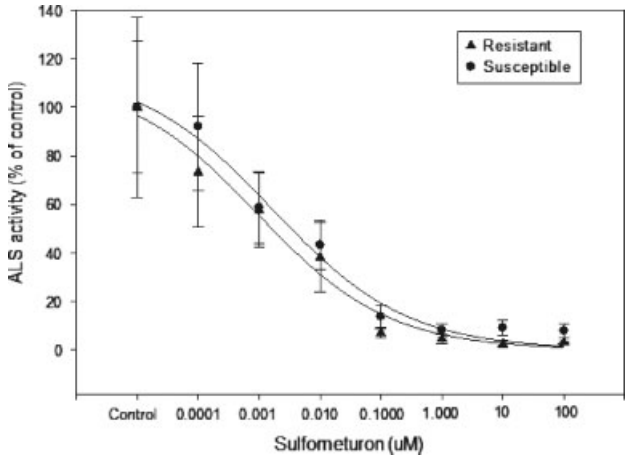

Figure 4. *In vitro* ALS enzyme activity in the presence of increasing concentrations of sulfometuron. Each data point represents the mean activity ± SE of three replicate treatments.

suffered by the resistant populations at high rates of sulfosulfuron; (2) the similar *in vitro* sensitivities of the ALS enzymes from S and R plants to sulfometuron; (3) reversal of sulfosulfuron resistance by malathion. While previous studies have suggested that plants with metabolism-based resistance to SU herbicides should remain susceptible to sulfometuron, as discussed in Yu *et al.*,[29] there are in fact some *L. rigidum* populations that survive sulfometuron but have no known ALS mutations (Yu Q, private communication). There are also at least two examples of potential cytochrome-P450-based SU resistance in *B. tectorum* populations from the United States. In one of these, cross-resistance to sulfosulfuron, accompanied by a decrease in biomass of the resistant plants and reversible with the addition of malathion, was selected with the use of primisulfuron.[30] In the second, a *B. tectorum* population with evolved resistance to four ALS inhibitors showed no difference in ALS enzyme sensitivity compared with a susceptible population.[31] Further work revealed that this population exhibited resistance owing partly to an enhanced metabolism mechanism, with evidence for the involvement of cytochrome P450 monooxygenases.[32]

Target-site ALS herbicide resistance in the six Western Australian *B. rigidus* populations characterised in the present study can be excluded because sulfometuron equally inhibited ALS in R and S plants (Fig. 4). The I_{50} in this study (average 0.0025 μM) was similar for other monocot species, with the *H. leporinum* ALS I_{50} for sulfometuron being 0.004 μM (Yu *et al.*[29]). Although other mechanisms of resistance (e.g. reduced herbicide uptake or translocation) cannot yet be discounted, the present results are consistent with previous studies identifying potential P450-based resistance.[30,33] Several studies with *L. rigidum* have shown that resistance in certain populations is likely to be metabolism based, involving cytochrome P450, although different P450 enzymes are probably involved for different herbicides.[21,28,34] A study using *Sinapis arvensis* L.[35] resistant to ethametsulfuron revealed that there were no differences in the resistant and susceptible biotypes with regard to herbicide absorption and translocation, and both biotypes had a sensitive ALS; however, the herbicide was metabolised more rapidly in the resistant biotype. Applying piperonyl butoxide, an inhibitor of cytochrome P450, decreased the rate of metabolism of ethametsulfuron in the resistant biotype, indicating that enhanced herbicide metabolism was the prominent mechanism of resistance.

The control of these ALS-resistant *B. rigidus* biotypes may initially be achieved by the use of the ALS IMI herbicides, as well as by other herbicide modes of action (Table 1). The development of IMI-tolerant wheat (Clearfield™) provides growers with the option to use IMI herbicides, which also have the advantage of controlling a number of other grass weed species.[10] Metribuzin can also be used selectively to control *Bromus* spp. in barley crops, although high rates may cause crop damage on some soil types.[36] Most importantly, weed numbers should be kept at a minimum through the use of integrated weed management options such as competitive crops and tools that deplete the soil weed seed bank, as resistance to some of the alternative herbicide control options has already been reported.[16–18]

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REFERENCES

1 Kon KF and Blacklow WM, *Bromus diandrus* Roth and *B. rigidus* Roth, in *The Biology of Australian Weeds. Vol. 1*, ed. by Groves R, Shepherd R and Richardson FJ. RG and FJ Richardson, Melbourne, Australia, pp. 13–27 (1995).

2 Kon KF and Blacklow WM, Identification, distribution and population variability of great brome (*Bromus diandrus* Roth) and rigid brome (*Bromus rigidus* Roth). *Aust J Agric Res* **39**:1039–1050 (1988).

3 Gill GS and Carstairs SA, Morphological, cytological and ecological discrimination of *Bromus rigidus* from *Bromus diandrus*. *Weed Res* **28**:399–405 (1988).

4 Gill GS and Blacklow WM, Variations in seed dormancy and rates of development of great brome, *Bromus diandrus* Roth., as adaptations to the climates of southern Australia and implications for weed control. *Aust J Agric Res* **36**:295–304 (1985).

5 Gill GS, Poole ML and Holmes JE, Competition between wheat and brome grass in Western Australia. *Aust J Exp Agric* **27**:291–294 (1987).

6 Cheam AH, The biology of intractable weeds: brome grass: a persistent but manageable weed. Farmnote No. 95/96, Department of Agriculture, Western Australia (2005).

7 Michael PJ, Owen MJ and Powles SB, Herbicide-resistant weed seeds contaminate grain sown in the Western Australian grainbelt. *Weed Sci* **58**:466–472 (2010).

8 Kleemann SGL and Gill GS, Differences in the distribution and seed germination behaviour of populations of *Bromus rigidus* and *Bromus diandrus* in South Australia: adaptations to habitat and implications for weed management. *Aust J Agric Res* **57**:213–220 (2006).

9 Chapman R, Bolger TP and Le Coultre IF, Seed dormancy release in three common pasture grasses from a Mediterranean-type environment under contrasting conditions. *Aust J Exp Agric* **39**:143–147 (1999).

10 Kleemann SGL and Gill GS, The role of imidazolinone herbicides for the control of *Bromus rigidus* (rigid brome) in wheat in southern Australia. *Crop Prot* **28**:913–916 (2009).

11 Bowran D, Weed control in wheat, in *The Wheat Book – Principles and Practice*, ed. by Anderson WK and Garlinge Jr. Agriculture Western Australia, Perth, Western Austrialia, pp. 248–253 (2000).

12 Saari LL, Cotterman JC and Thill DC, Resistance to acetolactate synthase inhibiting herbicides, in *Herbicide Resistance in Plants – Biology and Biochemistry*, ed. by Powles SB and Holtum JAM. Lewis Publishers, Boca Raton, FL, pp. 83–139 (1994).

13 Tranel PJ and Wright TR, Resistance of weeds to ALS-inhibiting herbicides: what have we learned? *Weed Sci* **50**:700–712 (2002).

14 Powles SB and Yu Q, Evolution in action: plants resistant to herbicides. *Annu Rev Plant Biol* **61**:317–347 (2010).

15 Heap IM, *International Survey of Herbicide Resistant Weeds*. [Online]. Available: http://www.weedscience.org [5 October 2011].

16 Boutsalis P and Preston CA, Resistance to acetyl-coenzyme A carboxylase (ACCase)-inhibiting herbicides in *Bromus* spp. in Australia, in *Proceedings of Fifteenth Australian Weeds Conference*, ed. by Preston C, Watts JH and Crossman ND. Weed Management Society of South Australia, Adelaide, Australia, pp. 538–540 (2006).

17 Ball DA and Mallory-Smith C, Sulfonylurea herbicide resistance in downy brome, in *Proceedings of Western Society of Weed Science*, ed. by Christianson K. Western Society of Weed Science, Las Cruces, NM, pp. 41–42 (2000).

18 Ball DA, Frost SM and Bennett LH, ACCase-inhibitor herbicide resistance in downy brome (*Bromus tectorum*) in Oregon. *Weed Sci* **55**:91–94 (2007).

19 Owen MJ and Powles SB, Distribution and frequency of herbicide-resistant wild oat (*Avena* spp.) across the Western Australian grain belt. *Crop Past Sci* **60**:25–31 (2009).

20 Christopher JT, Preston C and Powles SB, Malathion antagonizes metabolism-based chlorsulfuron resistance in *Lolium rigidum*. *Pestic Biochem Physiol* **49**:172–182 (1994).

21 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**:123–134 (1996).

22 Kreuz K and Fonné-Pfister R, Herbicide–insecticide interaction in maize: malathion inhibits cytochrome P450-dependent primisulfuron metabolism. *Pestic Biochem Physiol* **43**:232–240 (1992).

23 Seefeldt SS, Jensen JE and Fuerst EP, Log-logistic analysis of herbicide dose–response relationships. *Weed Technol* **9**:218–227 (1995).

24 Ray TB, Site of action of chlorsulfuron: inhibition of valine and isoleucine biosynthesis in plants. *Plant Physiol* **75**:827 (1984).

25 Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal Biochem* **2**:248–254 (1976).

26 Escorial C, Loureiro I, Rodríguez-García E and Chueca C, Population variability in the response of ripgut brome (*Bromus diandrus*) to sulfosulfuron and glyphosate herbicides. *Weed Sci* **59**:107–112 (2011).

27 Baerg RJ, Barrett M and Polge ND, Insecticide and insecticide metabolite interactions with cytochrome P450 mediated activities in maize. *Pestic Biochem Physiol* **55**:10–20 (1996).

28 Yu Q, Abdallah I, Han HP, Owen M and Powles S, Distinct non-target site mechanisms endow resistance to glyphosate, ACCase and ALS-inhibiting herbicides in multiple herbicide-resistant *Lolium rigidum*. *Planta* **230**:713–723 (2009).

29 Yu Q, Nelson JK, Zheng MQ, Jackson M and Powles SB, Molecular characterisation of resistance to ALS-inhibiting herbicides in *Hordeum leporinum* biotypes. *Pest Manag Sci* **63**:918–927 (2007).

30 Mallory-Smith C, Hendrickson P and Mueller-Warrant G, Cross-resistance of primisulfuron-resistant *Bromus tectorum* L. (downy brome) to sulfosulfuron. *Weed Sci* **47**:256–257 (1999).

31 Park KW and Mallory-Smith CA, Physiological and molecular basis for ALS inhibitor resistance in *Bromus tectorum* biotypes. *Weed Res* **44**:71–77 (2004).

32 Park KW, Fandrich L and Mallory-Smith CA, Absorption, translocation, and metabolism of propoxycarbazone-sodium in ALS-inhibitor resistant *Bromus tectorum* biotypes. *Pestic Biochem Physiol* **79**:18–24 (2004).

33 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**:258–261 (1999).

34 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**:1036–1043 (1991).

35 Veldhuis LJ, Linda M, O'Donovan JT, Dyer W and Hall JC, Metabolism-based resistance of a wild mustard (*Sinapis arvensis* L.) biotype to ethametsulfuron-methyl. *J Agric Food Chem* **48**:2986–2990 (2000).

36 Kleemann SGL and Gill GS, Applications of metribuzin for the control of rigid brome (*Bromus rigidus*) in no-till barley crops of southern Australia. *Weed Technol* **22**:34–37 (2008).