Herbicide cross resistance in *Bromus diandrus* and *B. rigidus* populations across southeastern Australia

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Summary Herbicide resistance to Group A (AC Case-inhibiting herbicides) and B herbicides (ALS-inhibiting herbicides) in *Bromus diandrus* and *B. rigidus* is becoming more common in southeastern Australia but there is limited information available on its regional distribution in either species. At present it is unclear if resistant brome populations differ in their response to different herbicides within the same herbicide group. Another unresolved question is related to the herbicide dose response of resistant brome populations. This study identified differences between Group A herbicides in their activity on resistant brome. However, increasing herbicide dose only marginally improved weed control in most cases. Varying degrees of Group B resistance was also detected in four brome populations. These initial findings suggest that increasing herbicide dose is unlikely to improve brome control but some Group A and B herbicides are more effective than others in controlling resistant populations.

Keywords Great brome, rigid brome, Group A herbicides, Group B herbicides, pot studies, mesosulfuron, pyroxsulam, imazapyr, imazapic, clethodim, haloxyfop, quizalofop-*P*-ethyl, fluazifop-*P*-butyl.

INTRODUCTION

Bromus diandrus (great brome) and *B. rigidus* (rigid brome) are significant weeds of broadacre agriculture across southern Australia. Both species are predominately found on lighter soils with *B. rigidus* more common in the lower-rainfall regions, whereas *B. diandrus* often occurs in higher rainfall cropping regions. A feature which makes both species difficult to control in cereals is staggered germination, in particular *B. rigidus* (Kleemann and Gill 2006). The increased adoption of reduced tillage in broadacre farming has increased the reliance on herbicides as the main tool to control brome. Pre-emergence herbicides such as trifluralin, pendimethalin and metribuzin are commonly used to control initial brome cohorts germinating with the crop. However, control of later emerging cohorts is often more difficult as the residual activity of pre-emergence herbicides decreases. Controlling both species in cereal crops with post-emergence herbicides is often difficult due to the limited choice of herbicides.

The main herbicides used in cereals are Group B herbicides (ALS-inhibiting herbicides) such as the sulfonylurea herbicides mesosulfuron (Atlantis), sulfosulfuron (Monza) and the sulfonamide herbicide pyroxsulam (Crusader). In addition the Group B imidazolinone herbicides imazapyr + imazapic (On-Duty), imazapyr + imazamox (Intervix) and imazapyr + imazapic + MCPA (Midas™) are only selective in Clearfield wheat and barley (Kleemann and Gill 2009). Unlike Group A and Group B imidazolinone herbicides which result in plant death, mesosulfuron and pyroxsulam often result in reduction of plant biomass and seed-set, with increased weed suppression in competitive crops. In broadleaf crops such as peas, beans, lentils and canola, Group A herbicides such as clethodim, haloxyfop, quizalofop and propaquizafop are commonly used. Overreliance on Group A and B herbicides to control brome has resulted in the occurrence of biotypes with resistance to these herbicides.

The incidence of herbicide resistance in brome across southeastern Australia was determined by random weed surveys followed by pot trials. In addition several biotypes with suspected resistance were tested for cross-resistance in dose response pot studies.

MATERIALS AND METHODS

Weed Survey Random weed surveys were conducted across southeastern Australia over 2006–2011 (Boutsalis *et al*. 2012). Amongst other weed species, brome was collected where identified in cropping fields (Boutsalis *et al*. 2012). The following autumn each brome sample was tested in outdoor pot trials. Ten seeds per 10 cm × 10 cm pot were sown in potting mix and grown outdoors. Each herbicide (26 g ai ha^{-1} haloxyfop, 48 g ai ha^{-1} clethodim and 9.9 g ai ha^{-1} mesosulfuron) treatment consisted of two replicate pots. Plants were sprayed at the Z12 stage with Group B herbicides and at the Z21 stage with Group A herbicides (Table 1). The herbicides were applied using a herbicide spray cabinet (output volume 109 L ha^{-1} water, pressure of 250 kPa, speed of 1 m s^{-1} using Tee-Jet 001 nozzles). All herbicides were used with their recommended adjuvants. After herbicide application the pots were returned outdoors the following day.

Dose response experiments Brome originating from twelve field populations, ten with suspected resistance was sown into pots in early September 2011 (Table 1). Ten seeds were sown into each 10 cm × 10 cm pot. At the appropriate stage, plants were treated with various doses of Group A or Group B herbicides in a cabinet sprayer (Table 2). The experiment was laid out in a completely randomised design with three replicates. Mortality data was analysed using PriProbit and the dose required to control 50% of the plants (LD_{50}) calculated. The LD_{50} ratio of resistant to susceptible populations for individual herbicides calculated.

RESULTS AND DISCUSSION

Random weed survey A series of random weed surveys over a 5-year period resulted in weed collections from most cropping areas across southeastern Australia (Figure 1, Table 3).

The incidence of randomly chosen fields containing brome in the entire survey areas ranged from 3 to 70% depending on the region (Figure 1, Table 3). Fields located in lower rainfall areas with lighter soils such as the upper Eyre Peninsula and Mallee regions contained significant levels of brome (Table 3).

Brome was less prevalent in fields located in the higher rainfall areas such as the south east of South Australia and northern Victoria. In lower rainfall areas, cropping rotations are generally less diverse with cereals being the dominant crop where brome is more difficult to control (Boutsalis *et al*. 2012). In contrast, the Yorke Peninsula, Wimmera and Victorian Mallee regions are more intensively cropped with a greater diversity of rotations and increased reliance on herbicides (Boutsalis *et al*. 2012). This is likely to be an important factor contributing to the presence of herbicide resistance in these regions (Table 3). The Group A and B herbicide resistance levels detected in brome were much lower than that observed in annual ryegrass across the same surveyed regions (Boutsalis *et al*. 2012). However, with the dominance of conservation tillage and the lack of effective herbicides in cereal crops, the incidence of brome across southeastern Australia has significantly increased (Kleemann and Gill 2006).

Resistance to Group A herbicides At the recommended field rates for fluazifop (70 g ai ha^{-1}), haloxyfop (39 g ai ha^{-1}), quizalofop (30 g ai ha^{-1}) and clethodim (48 g ai ha^{-1}) all plants from both susceptible brome populations (A496 & SE-1) were killed. In contrast, significant differences in herbicide resistance levels measured as LD_{50} ratios between Group A resistant brome populations was observed with some populations not controlled at even four times the field rate. The strongest resistance was observed to quizalofop and fluazifop with some populations exhibiting LD_{50} ratios greater than sixteen (Table 4). Haloxyfop and clethodim exhibited similar activity on resistant populations even though they belong to different chemistries. The practical implication is that haloxyfop and clethodim are likely to control a greater proportion of resistant individuals than quizalofop or fluazifop. However, further selection with continued application will quickly lead to significant resistance to both haloxyfop and clethodim.

Previous research has shown that certain ACCase target site mutations only confer resistance to FOP herbicides whereas other mutations can confer resistance to both FOP and DIM herbicides (Yu *et al*. 2007). As two out of the six FOP resistant populations were

Table 1. Locations of tested brome populations. Species D refers to *B. diandrus* and R refers to *B. rigidus*.

Sample ID	Species	Town	State
A228	R	Crystal Brook	SA
A306	D	Ultima	Vic
A496	D	Woomelang	Vic
A734	D	Mallee	Vic
A739.1	D	Ultima	Vic
A739.3	D	Ultima	Vic
A946.4	D	Yaapeet	Vic
1278.1	D	Yorke	WA
EP #12	R	Kimba	SA
EP #71	R	Yeelanna	SA
MN #29	D	Pt Broughton	SA
SE-1	D	Cooke Plains	SA

Table 2. Herbicide treatment details including adjuvants used and growth stage of brome populations used for the cross-resistance dose response experiments. Midas™ (Imazapic + Imazapyr + MCPA).

Herbicide	Rates (g ai ha^{-1})
Group A	
[1] Clethodim	24, 48, 96, 144, 192
[1] Quizalofop	20, 40, 80, 120, 160
[1] Haloxyfop	26, 39, 52, 91, 104
[1] Fluazifop	53, 106, 212, 318, 424
Group B	
[1] Mesosulfuron	9, 12, 15, 18, 27
[2] Pyroxsulam	15, 22.5, 30, 45, 60
[3] Midas™	9+3+115, 14+5+188, 20+7+260, 30+10+390, 40+13+519

[1] Hasten at 1% v/v;
[2] BS1000 at 0.25% v/v;
[3] Supercharge at 0.5% v/v

Figure 1. Map of surveyed regions across southeastern Australia showing the location of surveyed fields. Points represent each sampled field. Names in capitals are the main agronomic regions with the sub-regions in small font. Brome was not identified at all sites.

Table 3. Incidence of brome across South Australian and Victorian cropping regions and the level of herbicide resistance detected in each region to three herbicides.

Survey	Year	Fields surveyed	Fields with brome (%)	Haloxyfop (%)	Clethodim (%)	Mesosulfuron (%)
South Australia						
South East	2007	76	13	0	0	0
North Mallee	2007	82	54	0	0	0
South Mallee	2007	74	31	0	0	0
Mid North	2008	88	24	0	0	0
Yorke Peninsula	2008	122	23	4	4	4
Lower North	2008	61	18	0	0	0
Upper Eyre Peninsula	2009	104	44	0	0	3
Lower Eyre Peninsula	2009	75	35	0	0	2
Victoria						
North Central	2006	58	3	0	0	0
North East	2006	60	8	0	0	0
Mallee	2010	69	70	10	6	0
Wimmera	2010	80	44	6	3	0

not resistant to clethodim it indicates the presence of different mutations in different brome populations, although other mechanisms of resistance may also be involved.

Resistance to Group B herbicides The level of resistance to Group B herbicides was significantly lower than to Group A herbicides. Pyroxsulam at the recommended rate of 15 g ai ha^{-1} stunted brome with 30 g ai ha^{-1} required to severely inhibit both susceptible populations (data not shown). In contrast, four other populations exhibited resistance to pyroxsulam ranging from 2- to 4-fold resistance (Table 5). Brome samples resistant to pyroxsulam also exhibited resistance to mesosulfuron (Table 5). Resistance to Group B herbicides in self-pollinating grass weeds such as barley grass (Yu *et al*. 2007), wild oats and brome is a relatively recent phenomenon in Australian broadacre farming. In contrast, Group B resistance in wild oats and brome has been documented for over 20 years in the USA and Canada (Mallory-Smith *et al*. 1999, Friesen *et al*. 2000, Mengistu *et al*. 2003).

It is unlikely that resistance to Group B herbicides that are registered for brome control is entirely due to the repeated use of these herbicides. These herbicides have only been available for a short period (pyroxsulam) and their cost has restricted widespread use in marginal regions. It is possible that sulfonylurea herbicides such as chlorsulfuron, triasulfuron and metsulfuron, although not being registered for brome have imposed selection for Group B resistance. This phenomenon has been observed in barley grass which has evolved resistance to mesosulfuron from selection with other sulfonylurea herbicides which were registered for the control of other weed species (Yu *et al*. 2007).

Resistance to Imazapic + imazapyr + MCPA (Midas™) was also detected at similar levels to mesosulfuron and pyroxsulam (Table 5). However, the Western Australian population 1278.1 which was resistant to both pyroxsulam and mesosulfuron was not cross-resistant to Midas™ (Table 5). This response indicates either a different mechanism of resistance, or the presence of a different Group B target site resistance mutation. Nandula and Messersmith (2000) identified that resistance to imazamethabenz-methyl in wild oats was due to enhanced metabolism. Resistance to sulfonylurea herbicides but not imidazolinone herbicides has been reported in ryegrass (Burnet *et al*. 1994) and barley grass (Yu *et al*. 2007). In contrast, populations MN#29, EP#71 and EP#12 exhibited resistance to Midas™ indicating that control in the field would be poor (Table 5). Ryegrass populations resistant to sulfonylurea and imidazolinone herbicides have been identified for over 20 years (Christopher *et al*. 1992) with barley grass also recently reported as being resistant to both herbicide classes (Owen *et al*. 2012).

Table 4. The LD_{50} ratio of each resistant population compared to susceptible populations. Herbicide abbreviations are CL (clethodim), QZ (quizalofop), FL (fluazifop), HX (haloxyfop).

Population	Herbicide			
	CL	QZ	FL	HX
A496	1	1	1	1
A228	10	16	>16	7
A306	4	11	15	3
A734	1	16	1	3
A739.1	7	16	16	3
A739.3	6	10	>16	3
A946.4	1	8	4	1.5
SE-1	1	1	1	1

Table 5. The LD_{50} ratio of each resistant population compared with the susceptible population. Herbicide abbreviations are pyroxsulam (PM), mesosulfuron (MN) and Midas™.

Population	Herbicide		
	PM	MN	#Midas™
MN #29	4	5	3
EP #12	3.5	5	3
EP #71	2	5	3
A496	1	1	1
1278.1	3	4	1
A946.4	1	1	1

Imazapic + Imazapyr + MCPA

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