The extent of herbicide resistance in Lolium rigidum Gaud. (annual ryegrass) across south-eastern Australia as determined from random surveys

Authors: Broster, John, Boutsalis, Peter, Gill, Gurjeet S., and Preston, Christopher

Source: Crop and Pasture Science, 73(11) : 1308-1317

Published By: CSIRO Publishing

URL: https://doi.org/10.1071/CP21753

RESEARCH PAPER
https://doi.org/10.1071/CP21753

CROP & PASTURE SCIENCE

The extent of herbicide resistance in *Lolium rigidum* Gaud. (annual ryegrass) across south-eastern Australia as determined from random surveys

John Broster[A,*], Peter Boutsalis[B], Gurjeet S. Gill[B] and Christopher Preston[B]

For full list of author affiliations and declarations see end of paper

***Correspondence to:**
John Broster
Gulbali Institute for Agriculture Water Environment, Charles Sturt University, Boorooma Street, Wagga Wagga, NSW 2678, Australia
Email: jbroster@csu.edu.au

Handling Editor:
Mary-Jane Rogers

ABSTRACT

Context. Annual ryegrass (*Lolium rigidum*) is a major weed of crop production in southern Australia that readily develops resistance to herbicides. Resistance increases both yield losses and control costs associated with this species. **Aims.** This study aimed to gauge the extent and distribution of resistance to herbicides in *L. rigidum* across south-eastern Australian grain production systems by collecting seed from randomly selected fields. **Methods.** A total of 1441 weed populations were collected through random surveys conducted over 5 years across 13 agricultural regions of four states with these samples then tested for resistance to eight herbicides from six modes of action. **Key results.** Resistance to diclofop-methyl and sulfometuron-methyl was most common, being present in 64% and 63% of populations respectively. Glyphosate resistance was present in 4% of populations collected. Only 15% of populations collected were susceptible to all herbicides tested. Large differences in resistance occurred between the 13 regions surveyed with resistance to diclofop-methyl ranging from 15% to 86% of populations and sulfometuron-methyl from 12% to 96%. Resistance to post-emergent herbicides tended to be higher than pre-emergent herbicides. Multiple resistance was common with 60% of populations collected having resistance to two or more herbicide modes of action. **Conclusions.** There were significant differences in the extent of multiple resistance in *L. rigidum* populations collected from individual regions suggesting that the rates of resistance evolution have differed between regions. **Implications.** The high incidence of herbicide resistance in *L. rigidum* populations randomly collected from south-eastern Australian cropping fields highlights the need for the adoption of additional weed control practices to mitigate the impact of this species on grain production systems.

Keywords: ACCase-inhibiting herbicide, ALS-inhibiting herbicide, diclofop-methyl, glyphosate, multiple resistance, pre-emergent herbicide, ryegrass, sulfometuron-methyl.

Received: 5 November 2021
Accepted: 26 April 2022
Published: 24 May 2022

Cite this:
Broster J *et al.* (2022)
Crop & Pasture Science, **73**(11), 1308–1317.
doi:10.1071/CP21753

Introduction

Lolium rigidum Gaud. (annual ryegrass) is a major weed of crops in south-eastern Australia accounting for an estimated AUD93 million per annum in yield loss and considerably more in control costs (Llewellyn *et al.* 2016). The adoption of no-till farming across this region over the past 20 years has resulted in the reliance on herbicides for weed control (Llewellyn *et al.* 2012). Extensive resistance to post-emergent herbicides, especially those inhibiting the acetyl Coenzyme A carboxylase (ACCase) and acetolactate synthase (ALS) enzymes, has resulted in the widespread adoption of pre-emergent herbicides for weed control in cereal crops (Boutsalis *et al.* 2014). More recently, *L. rigidum* populations from south-eastern Australia have evolved resistance to many of these pre-emergent herbicides (Boutsalis *et al.* 2012; Brunton *et al.* 2018).

L. rigidum is well adapted to Australian winter cropping region environments and production systems. In the largely Mediterranean-type climate of this region, this weed germinates with the onset of the growing season in late autumn and sets seed at crop maturation in late spring (Gill 1996). While individual *L. rigidum* plants are not highly competitive, left uncontrolled this species can quickly establish large populations that can cause near complete yield loss (Reeves 1976; Lemerle *et al.* 1995).

One of the challenges in managing herbicide resistant populations of *L. rigidum* is understanding the extent of resistance present in individual fields. This can be achieved by testing for resistance by farmers; however, this is frequently not done due to impracticality of seed collection during harvest, cost and other factors (Llewellyn *et al.* 2002; Boutsalis and Broster 2006; Broster *et al.* 2019*a*). A more accurate and unbiased method to determine the incidence of herbicide resistance is through random surveys. Previous surveys have indicated that although resistance is extensive and widespread across south-eastern Australia, it is not uniformly distributed (Broster *et al.* 2011, 2012, 2013; Boutsalis *et al.* 2012). In some regions, resistance to one or more herbicides is much less frequent, providing farmers in these regions with greater opportunities to use herbicides for weed control.

The wide distribution of resistance to herbicides, particularly to post-emergent herbicides, in *L. rigidum* in Australia and the subsequent lack of in-crop selective herbicides with alternative modes of action, has resulted in the widespread adoption of recently released pre-emergent herbicides for the control of this weed (Boutsalis *et al.* 2014). These herbicides, prosulfocarb + S-metolachlor introduced in 2008 and pyroxasulfone introduced in 2012, have become commonly used for *L. rigidum* control in cereals in many regions across south-eastern Australia (Brunton *et al.* 2019). Due to this increased selection pressure, these herbicides are under considerable threat from resistance evolution.

The aim of this study was firstly to gauge the extent and distribution of resistance to herbicides in *L. rigidum* in south-eastern Australia by collecting seed from randomly selected fields, and secondly to compare the findings to those of previous surveys. Populations collected were tested with eight herbicides, from six modes of action, that are registered for the control of *L. rigidum* in Australian winter cropping systems, including pre-emergent herbicides introduced since previous surveys were conducted.

Materials and methods

Seed collection

Weed seed collection surveys of South Australia (SA), Victoria (Vic.), Tasmania (Tas.) and southern New South Wales (NSW) cropping regions were conducted at crop maturity in the 2013–2017 growing seasons (Fig. 1). Fields were selected by travelling for a predetermined distance (5 or 10 km depending upon the size of surveyed area). At each stop, a single field was surveyed with sampling commencing 10 m in from the edge of the crop and continued in a zig zag pattern on a transect that covered at least one ha of the field. The sampling location (longitude and latitude) was recorded using a global positioning system (GPS) navigational unit. After collection the populations were kept in a covered enclosure where they were subjected to fluctuating summer temperatures for 4 months, after which time the seed was threshed from the heads and stored at room temperature until tested. A full description of the surveying methodology is described in Boutsalis *et al.* (2012).

As *L. rigidum* becomes mature at a similar time across this very large area, it is impractical to survey it in a single year. Therefore, the area was divided into 13 regions and two to three regions were surveyed each year (Fig. 1). There was a target of assessing at least 120 crop fields in each region, collecting seed from *L. rigidum* populations if present.

Plant growth and treatment

Populations collected from SA and Vic. were tested at the Waite Campus, University of Adelaide. Populations collected from Tas. and NSW were tested at Charles Sturt University (CSU), Wagga Wagga. Each testing centre used their own standard susceptible population (Adelaide – SLR4 and VLR1; CSU – 120327a), which was included in every test. A standard resistant population was also included where one was available for that herbicide.

Seed planting, seedling maintenance and herbicide screening procedures are described in Boutsalis *et al.* (2012) and Broster *et al.* (2011). In brief, 50–60 seeds (0.2 grams) of seed were sown into pots (three replicates at CSU, one replicate at Adelaide), with germination of plants to be treated with post-emergent herbicides counted before treatment; at this stage populations at CSU were thinned to 20 plants (not thinned at Adelaide). At CSU seeds were sown into plastic trays (150 mm × 100 mm × 60 mm) filled with either a 50:50 peat:sand mix (diclofop-methyl, clethodim and glyphosate) or garden loam (all other herbicides) while at Adelaide 0.55 L pots containing a coco peat mix were used. Seeds treated with pre-emergent herbicides were sown on the soil surface, sprayed and then covered with either 5 mm of garden loam (CSU) or coco peat mix (Adelaide). At CSU trials were conducted in a temperature-controlled glasshouse (10°C–25°C) while at Adelaide they were conducted outdoors under natural autumn and winter growing conditions. At both locations the trials were watered daily and fertilised as required.

The herbicides and rates used in the testing are listed in Table 1. Sulfometuron-methyl was used as the sulfonylurea herbicide in this study, as it provided more reliable results in pots than did other sulfonylurea herbicides.

Plants were counted as survivors of the herbicide if they had emerged and developed to the two-leaf stage 28 days after treatment with pre-emergent herbicides or if they had new shoot growth 21 days after treatment with

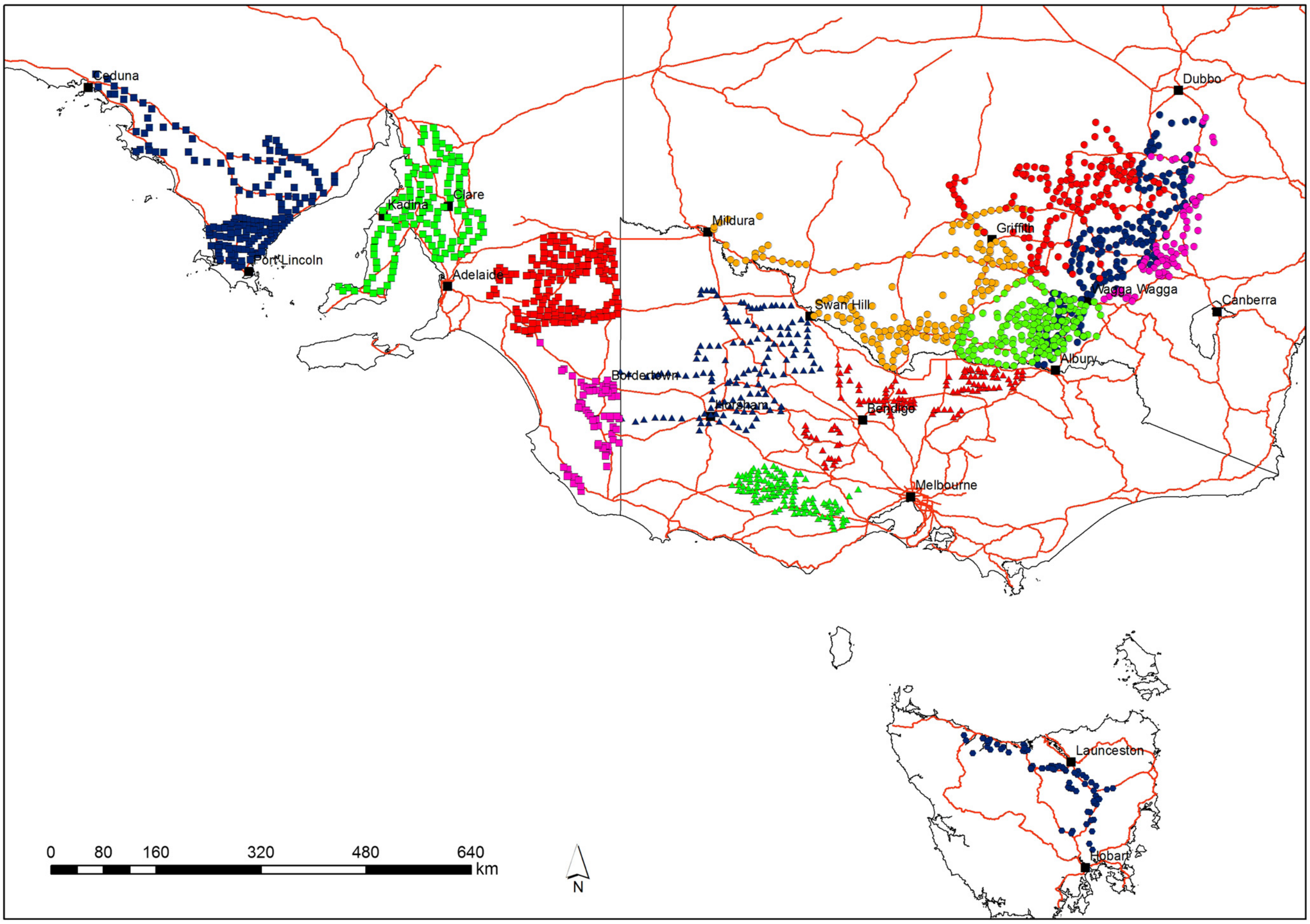

Fig. 1. Survey area across south-eastern Australia. Each point represents a field surveyed in this work. The 13 regions are designated by different symbols and colours: SA Eyre Peninsula (blue squares); SA Mid-north (green squares); SA Mallee (red squares); SA South-east (pink squares); Vic. Wimmera Mallee (blue triangles); Vic. Southern (green triangles); Vic. North-east (red triangles); NSW Western (orange circles); NSW Plains (red circles); NSW Southern (green circles); NSW Slopes (blue circles); NSW Eastern (pink circles); and Tas. (blue hexagons).

Table 1. Herbicides, suppliers and rates used for testing of *L. rigidum* populations.

Herbicide	Chemistry	Rate (g a.i. or a.e. ha^{-1})[A]	Trade name	Supplier
Diclofop-methyl	ACCase inhibitor (FOP[B])	375	Diclofop-methyl	Cheminova Australia
Clethodim	ACCase inhibitor (DIM[C])	120	Havoc	Nufarm Australia
Sulfometuron-methyl	ALS inhibitor (Sulfonylurea)	15	Oust	DuPont Australia
Imazamox + imazapyr	ALS inhibitor (Imidazolinone)	24.75 + 11.25	Intervix	BASF Australia
Glyphosate	EPSPS[D] inhibitor	540	Weedmaster Argo	Nufarm Australia
Trifluralin	Dinitroaniline	800	TriflurX	Nufarm Australia
Prosulfocarb + S-metolachlor	Thiocarbamate + Isoxazoline	2000 + 300	Boxer Gold	Syngenta Australia
Pyroxasulfone	Isoxazoline	100	Sakura	Bayer CropScience

[A]g a.i., grams active ingredient; a.e. ha^{-1}, acid equivalent per hectare.
[B]FOP, aryloxyphenoxypropionate.
[C]DIM, cyclohexanedione.
[D]EPSPS, 5-enolpyruvylshikimate-3-phosphate.

post-emergent herbicides. Following Boutsalis *et al.* (2012), populations from each field were considered resistant only if 20% or more of the individuals in that population had survived the herbicide application.

Statistical analysis

The frequency of resistance to each herbicide in each region was compared to the average frequency across all regions using an exact binomial test (McDonald 2014) with the null hypothesis being that resistance was equally distributed across regions. The same test was used to compare frequencies of resistance in SA, Vic., Tas., NSW Slopes and NSW Plains regions with previous surveys with the null hypothesis that resistance frequencies had not changed between surveys.

For ACCase- and ALS-inhibiting herbicides, where two different herbicides were tested within each mode of action, due to the large number of samples a multinomial goodness of fit test (G statistic) was used to test if the frequency of resistance to these two modes of action was the same (McDonald 2014) with the null hypothesis being that resistance occurred equally across the mode of action. This test was also used to compare the distribution of resistance to cumulative numbers of modes of action in each region with the average distribution across all the populations (McDonald 2014). In this case the null hypothesis was that resistance in each region had evolved at similar rates and so the proportion of populations with resistance to different numbers of modes of action should be similar.

Results

Populations collected and crop distribution

Over the period 2013–2017, 1760 crop fields were surveyed and 1441 *L. rigidum* populations collected, with *L. rigidum* present in 82% of fields surveyed. Wheat was the dominant crop in all the cropping regions, occurring on average in 64% of the surveyed fields. The next most commonly occurring crop was barley in 14% of fields, then canola in 8% and pulse crops (field peas, lentils, faba beans, lupins and chickpeas) in 6% of fields (Table 2).

Resistance to post-emergent selective herbicides

Resistance to ACCase inhibiting herbicides was present in *L. rigidum* populations collected in all regions (Table 3). Sixty four percent of populations across the surveyed area were resistant to the ACCase inhibitor diclofop-methyl with differences between regions. The NSW Western region (15%, $P < 0.0001$), SA Mallee region (22%, $P < 0.0001$) and SA Eyre Peninsula (47%, $P < 0.0001$) had fewer resistant populations compared to the average, whereas SA Mid-north (74%, $P = 0.015$), NSW Eastern region (77%, $P = 0.038$), SA South-east region (84%, $P < 0.0001$), NSW Slopes region (85%, $P < 0.0001$), NSW Southern region (85%, $P < 0.0001$)

Table 2. Number of fields visited, distribution of crops present and *L. rigidum* populations collected in random seed collection surveys at the end of the winter growing season by region and year.

Region	Year surveyed	Fields visited (No.)	*L. rigidum* populations (No.)	Crops				
				Wheat	Barley	Canola	Pulse	Other
				Fields sampled (%)				
SA								
Eyre Peninsula	2014	167	145	70	9	14	4	3
Mid-north	2013	150	136	63	21	7	9	0
Mallee	2017	164	102	58	31	4	4	3
South East	2017	74	65	52	8	5	21	14
Vic.								
Wimmera Mallee	2015	140	122	58	24	2	15	1
Southern	2014	116	116	61	8	29	2	0
North-east	2016	122	115	71	16	7	6	3
NSW								
Western	2015	164	117	50	19	7	4	20
Plains	2016	152	115	64	19	1	1	15
Southern	2017	162	128	60	8	15	4	13
Slopes	2013	199	163	87	7	0	4	2
Eastern	2014	75	65	63	0	3	3	21
Tas.	2014	75	52	52	13	0	4	31
Total		1760	1441	64	14	8	6	8

Table 3. Extent of resistance to various herbicides of populations collected from the 13 different regions of southern Australia. Populations were considered to be resistant if >20% of individuals within that population survived application of the herbicide.

Herbicide	SA				Vic.			NSW					Tas.	Mean
	EP	MN	ML	SE	WM	S	NE	W	PL	SL	S	E		
	Populations resistant (%)													
Diclofop-methyl	47	74	22	84	70	86	72	15	65	85	85	77	42	64
Clethodim	4	7	1	19	3	3	1	1	1	12	3	16	4	5
Sulfometuron-methyl	80	71	54	66	60	96	74	29	35	60	74	70	12	63
Imazamox + Imazapyr	47	83	37	52	31	33	51	7	39	39	75	81	16	47
Glyphosate	1	1	2	27	7	4	3	5	0	2	7	0	0	4
Trifluralin	34	66	39	41	31	6	0	2	0	21	1	0	0	20
Prosulfocarb + S-metolachlor	1	0	0	5	0	0	0	0	0	0	0	0	0	0.3
Pyroxasulfone	0	0	1	5	0	0	0	0	0	0	0	0	0	0.3

EP, Eyre Peninsula; MN, Mid-north; ML, Mallee, SE, South-east; WM, Wimmera Mallee; S, Southern; NE, North-east; W, Western; PL, Plains; SL, Slopes; E, Eastern.

and Vic. Southern region (86%, $P < 0.0001$) all had more resistant populations compared to the average (Table 3).

Compared to previous surveys of resistance in *L. rigidum* in SA, Vic., Tas., NSW Slopes region and NSW Plains region, the recent survey shows increases in the frequency of resistance to ACCase-inhibiting herbicides in many of the regions surveyed (Broster *et al.* 2011, 2012, 2013; Boutsalis *et al.* 2012). For example, in 2005 35% of populations had resistance to diclofop-methyl in Vic. Wimmera Mallee region (Boutsalis *et al.* 2012) compared to 70% of populations in 2015 ($P < 0.0001$) (Table 4). Likewise, resistance to this herbicide was detected in 14% of populations from Tas. in 2010 (Broster *et al.* 2012) compared to 42% in 2014 ($P < 0.0001$). Resistance to diclofop-methyl also increased in the other regions except SA Mid-north region ($P = 0.617$) and SA South-east region ($P = 0.067$), where resistance was already high (Boutsalis *et al.* 2012) (Table 5).

Although clethodim is also an ACCase inhibitor, the frequency of resistant populations (5% overall) to this herbicide was lower than to diclofop-methyl (G = 2304; $P < 0.0001$). The incidence of resistance to clethodim also varied between regions with the NSW Slopes (12%, $P = 0.0016$), NSW Eastern (16%, $P = 0.0012$) and SA South-east regions (19%, $P < 0.0001$) all having more resistant populations than the average (Table 3). Despite the high frequency of resistance to diclofop-methyl, clethodim remains commonly used for control of *L. rigidum* in canola and pulse crops in southern Australia due to a much lower frequency of resistant populations (Saini *et al.* 2015*b*).

Across the surveyed area 63% of populations were resistant to the sulfonylurea herbicide, sulfometuron-methyl with significant differences between regions. Tas. (12%, $P < 0.0001$), NSW Western region (29%, $P < 0.0001$) and NSW Plains region (35%, $P < 0.0001$) all had fewer

Table 4. Increase in FOP, SU and trifluralin percentage resistance since the regions were last surveyed (Broster *et al.* 2011, 2012, 2013; Boutsalis *et al.* 2012).

	FOP			SU			Trifluralin		
	Latest survey	Previous survey	*P* value	Latest survey	Previous survey	*P* value	Latest survey	Previous survey	*P* value
SA Eyre Peninsula	47	30	<0.0001	80	78	0.617	34	5	<0.0001
SA Mid-north	74	76	0.617	71	73	0.699	66	40	<0.0001
SA Mallee	22	6	<0.0001	54	67	0.006	39	19	<0.0001
SA South-east	84	60	0.067	66	69	0.595	41	39	0.704
Vic. Wimmera Mallee	70	35	<0.0001	60	57	0.583	31	5	<0.0001
Vic. Southern	86	79	0.067	96	88	0.009	6	0	<0.0001
Vic. North-east	72	40	<0.0001	74	43	<0.0001	0	2	0.18
Tas.	42	14	<0.0001	12	14	0.841	0	0	1.0
NSW Slopes	85	70	0.0001	60	49	0.013	21	2	<0.0001
NSW Plains	65	43	<0.0001	35	41	0.185	0	0	1.0

FOP, aryloxyphenoxypropionate; SU, sulfonylurea.

Table 5. Extent of resistance to multiple herbicides in *L. rigidum* from each of the 13 surveyed areas across south-eastern Australia.

Modes of action with resistance	SA				Vic.			NSW					Tas.
	EP	MN	ML	SE	WM	S	NE	W	PL	SL	S	E	
	Populations (% of tested)												
0	12.3	2.9	17.2	1.7	11.6	1	12.5	55.1	32.6	6.7	5.9	12.1	45.2
1	38.5	8.7	51.6	16.7	25.6	14	27.1	25.6	28.4	20.9	20	15.5	35.7
2	31.1	32.7	15.1	33.3	39.5	80	58.3	17.9	38.9	56.7	68.2	72.4	19
3	16.4	51.9	14	31.7	20.9	5	2.1	1.3	0	15.7	5.9	0	0
4	1.6	3.8	2.2	15	2.3	0	0	0	0	0	0	0	0
5	0	0	0	1.7	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0
G statistic	14.3	103	44.6	54.4	2.36	61.8	21.8	82.2	44.4	18	23.8	28.9	38.6
P	0.026	<0.0001	<0.0001	<0.0001	0.88	<0.0001	0.001	<0.0001	<0.0001	0.006	0.0006	0.0001	<0.0001

The multinomial goodness of fit tests (G statistic) compared the distribution of resistance to modes of action for each region with the average distribution across south-eastern Australia.

EP, Eyre Peninsula; MN, Mid-north; ML, Mallee, SE, South-east; WM, Wimmera Mallee; S, Southern; NE, North-east; W, Western; PL, Plains; SL, Slopes; E, Eastern.

resistant populations to sulfometuron-methyl than the average whereas NSW Southern region (74%, $P = 0.002$), Vic. North-east region (74%, $P = 0.012$), SA Eyre Peninsula region (80%, $P < 0.0001$) and Vic. Southern region (96%, $P < 0.0001$) all had more resistant populations than the average (Table 3). Imazamox + imazapyr is a common herbicide mix used for weed control in imidazolinone tolerant (Clearfield®) crops in southern Australia and is a different class of ALS-inhibiting herbicide to the sulfonylurea herbicides (Tranel and Wright 2002). Resistance to imazamox + imazapyr (47% overall) was lower ($G = 150$; $P < 0.0001$) than for the sulfonylurea herbicides. Resistance to imazamox + imazapyr varied across the regions (Table 3). The NSW Western region (7%, $P < 0.0001$), Tas. (16%, $P < 0.0001$) Vic. Wimmera Mallee region (31%, $P = 0.001$) and Vic. Southern region (33%, $P = 0.0038$) all had fewer resistant populations to this herbicide mixture compared to the average. NSW Southern region (75%, $P < 0.0001$), NSW Eastern region (81%, $P < 0.0001$) and SA Mid-north region (83%, $P < 0.0001$) all had more resistant populations than the average (Table 3).

In previous surveys, resistance to sulfonylurea herbicides was high in most regions (Broster *et al.* 2011, 2012; Boutsalis *et al.* 2012). In the current survey, frequencies of resistance to sulfonylurea herbicides remained similar to previous surveys in most regions with the exception of SA Mallee ($P = 0.006$) where the frequency decreased and, Vic. North-east ($P < 0.0001$), Vic. Southern ($P = 0.009$) and NSW Slopes ($P = 0.013$), where resistance frequency had increased (Table 4). Weeds resistant to the sulfonylurea herbicides often remain susceptible to imidazolinone herbicides (Yu and Powles 2014). However, resistance to the imidazolinone herbicides in *L. rigidum* has reached high levels across much of south-eastern Australia (Table 3). In some regions, the level of resistance to imidazolinone herbicides was much lower than resistance to sulfonylureas but in other regions, *L. rigidum* populations were equally resistant to both herbicides.

Resistance to glyphosate

Across the surveyed area only 4% of populations were glyphosate resistant (Table 3). There was variation in resistance across the regions with the SA South-east region having more resistant populations to glyphosate (27%, $P < 0.0001$) compared to the average, while several regions (NSW Plains, NSW Eastern, Tas.) had no resistant populations (Table 3).

Resistance to pre-emergent herbicides

Resistance to the pre-emergent herbicides was present in 20% of populations; however, the frequency of resistant populations was variable between regions (Table 3). Resistance to trifluralin was higher than the average in the SA Mid-north region (66%, $P < 0.0001$), SA South-east region (41%, $P < 0.0001$), SA Mallee region (39%, $P < 0.0001$), SA Eyre Peninsula region (34%, $P < 0.0001$) and Vic. Wimmera Mallee region (31%, $P = 0.002$) (Table 3). All other regions, except NSW Slopes, had fewer resistant populations than the average, with no resistance detected in Vic. North-east, NSW Western, NSW Plains or Tas. regions (Table 3). The incidence of resistance to the two newer pre-emergent herbicides, prosulfocarb + S-metolachlor and pyroxasulfone, was low, between 0 and 5% of populations. Resistance to these two herbicides was only found in two of the 12 surveyed regions, with only the SA South-east region having more resistant populations to both herbicides compared with the average ($P = 0.001$ for both herbicides) (Table 3).

Trifluralin resistance increased from 2% in NSW Slopes in 2007 (Broster *et al.* 2011) compared with 21% in this survey (P < 0.0001). Likewise, trifluralin resistance increased from 5% of populations from the SA Eyre Peninsula in 2009 (Boutsalis *et al.* 2012) to 47% in 2014 (P < 0.0001) (Table 4). However, trifluralin resistance had not changed in SA South-east (P = 0.704), Vic. North-east (P = 0.18), NSW Plains (P = 1) and Tas. regions (P = 1) compared with previous surveys (Boutsalis *et al.* 2012; Broster *et al.* 2012, 2013) (Table 4).

Multiple resistance

Across south-eastern Australia, 15% of the populations were susceptible to all herbicides tested. A further 25% of populations were resistant to a single mode of action and the remaining 60% of populations were resistant to multiple herbicide modes of action (Fig. 2). Most common was resistance to two modes of action in 45% of populations (Fig. 2). No population had resistance to all six herbicide modes of action tested; however, there was one population with resistance to five modes of action and 18 populations with resistance to four modes of action.

The extent of multiple resistance was variable across the 13 regions sampled (Table 4). SA Mid-north, SA South-east, Vic. Southern, NSW Southern and NSW Slopes regions all had less than 10% of populations with susceptibility to all herbicides. In contrast, 55% of populations from NSW Western region were completely susceptible. The amount of multiple resistance varied from 19% in Tas. and NSW Western region to 88% in SA Mid-north region. SA South-east region had one sample with resistance to five modes of action and all regions of SA and Vic. Wimmera Mallee region had populations with resistance to at least four modes of action, compared with none of the regions in NSW or Tas. (Table 5). The multinomial goodness of fit test (Table 5) confirmed the differences between regions with only the Vic.

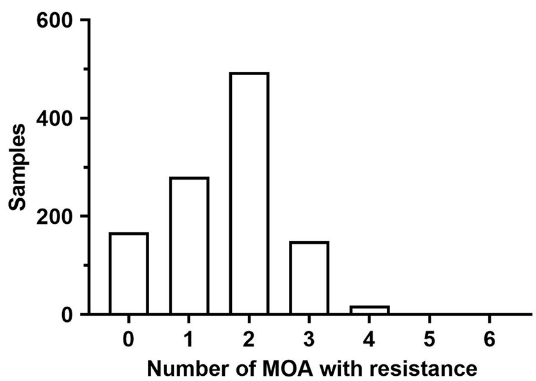

Fig. 2. Number of populations with resistance to different numbers of herbicide modes of action (MOA) in *L. rigidum* in south-eastern Australia. Only populations tested with all eight herbicides from six herbicide modes of action were included in the analysis.

Wimmera Mallee region having a distribution similar to south-eastern Australia as a whole.

Overall, 60% of *L. rigidum* populations from south-eastern Australia displayed resistance to two or more herbicide modes of action (Fig. 2). This is a modest increase in multiple resistance from 51% detected a decade previously (Boutsalis *et al.* 2012). In NSW, the level of multiple resistance had increased in NSW Plains region (19% to 39% $P \leq 0.0001$) (Broster *et al.* 2013), but there was little change for NSW Slopes (72% this survey P = 0.314) as multiple resistance was already at 76% of populations previously (Broster *et al.* 2011).

Discussion

The random collection and herbicide screening of more than 1400 *L. rigidum* populations, across an estimated 9.5 million ha, represents an extensive herbicide resistance survey of south-eastern Australian cropping regions. *L. rigidum* resistance to herbicides registered for control of this weed in cropping systems is widespread. The proportion of populations that were resistant to at least one herbicide ranged from 54% in Tas. to 99% in Vic. Southern. Resistance to the post-emergent ALS and ACCase-inhibiting herbicides was most common; however, resistance was also present to the three pre-emergent herbicides tested. The extent of resistance detected provides a challenge for both current and future control of *L. rigidum* in the regions' cropping systems.

Lolium spp. overseas are also prone to developing resistance. A survey of 75 *L. perenne* ssp. *multiflorum* (Lam.) Husnot (Italian ryegrass) populations from Oregon identified resistance to diclofop-methyl in 59% of populations, to the sulfonylurea herbicides in 27% of populations and resistance to the imidazolinone herbicides in 11% of populations (Rauch *et al.* 2010). This would be expected as similar crops are grown in both regions (Oregon and south-eastern Australia); therefore it would be expected that herbicide options and use would also be similar.

While diclofop-methyl resistance has increased, clethodim resistance is still below 10% of populations in the majority of regions (Table 3). This is consistent with other studies, in Australia and overseas, that have shown populations of *L. rigidum* resistant to diclofop-methyl and other FOP herbicides are often controlled by DIM herbicides (Yu *et al.* 2007; Rauch *et al.* 2010; Saini *et al.* 2015*a*).

A 2015 survey of 348 *L. rigidum* populations from Western Australia identified 79% with resistance to diclofop-methyl, 92% with resistance to sulfometuron-methyl and 14% resistant to clethodim (Owen and Powles 2018) compared with the average for south-eastern Australia of 64% resistant to diclofop-methyl, 63% resistant to sulfometuron-methyl and 5% resistant to clethodim (Table 3). In contrast, the incidence of resistance to trifluralin was 20% across south-eastern Australia, compared to 1% in Western Australia (Owen *et al.* 2014; Owen and Powles 2018). These differences reflect interactions between soil type, management practices and crops grown, all of which influence the choice of herbicides and frequency of use of the different herbicides (Broster *et al.* 2019*b*).

In comparison with trifluralin the frequency of resistance to prosulfocarb and pyroxasulfone is lower (≤5%). This was expected as trifluralin has a longer history of use across the surveyed area (Boutsalis *et al.* 2006); however the detection of resistance to prosulfocarb and pyroxasulfone after a relatively short exposure is a cause for concern. Therefore, it is recommended that growers should consider adopting management practices (e.g. herbicide mixtures) that can slow down resistance evolution, prolonging the life of these herbicides.

The 5-enolpyruvylshikimate-3-phosphate synthase inhibitor glyphosate is one of the most important herbicides used for weed control globally and in Australia (Duke and Powles 2008). Despite the widespread use of glyphosate in farming systems of south-eastern Australia, glyphosate resistance was rare, except in SA South-east region, where it was present in 27% of fields. Glyphosate resistance was also identified in 1% of *L. rigidum* populations found in Western Australia (Owen *et al.* 2014).

While *L. rigidum* in Australia was globally the first weed species to evolve resistance to glyphosate in 1996 (Powles *et al.* 1998; Pratley *et al.* 1999), glyphosate resistance in Australia is still lower than in several weed species of North America (Heap and Duke 2018). A survey of *Kochia scoparia* (L) Roth (kochia) populations in Alberta found 13 glyphosate resistant populations out of 300 sites sampled (Hall *et al.* 2014). In contrast, a survey of *Amaranthus rudis* Sauer (waterhemp) populations in Missouri found 30% resistant to glyphosate (Schultz *et al.* 2015). Canola is the only glyphosate resistant winter crop grown across the surveyed area, and during the period reported in this paper comprised no more than 17% of either NSW or Victorian canola crops (Agricultural Biotechnology Council of Australia 2022), with it unable to be grown in South Australia or Tasmania due to government moratoria. This compares with over 90% of canola crops in Canada having herbicide resistant cultivars, mainly glyphosate resistant, in 2006 (O'Donovan *et al.* 2006) and 91% of soybeans being herbicide tolerant by 2007 (Bonny 2008). As such, it would be expected that there would be both less applications of glyphosate and more of other herbicides, reducing selection pressure for the development of glyphosate resistance.

There were differences between the regions in resistance to multiple herbicide modes of action (Table 4), indicating that resistance evolution is not occurring at the same rates across the regions. These differences are likely to result from differences in crop rotations, including pastures (Table 2), and management practices reducing reliance on individual herbicide modes of action for *L. rigidum* control (Broster *et al.* 2019*b*). While multiple resistance often occurs through the sequential use of different herbicides, it can also occur through cross-resistance, predominantly through enhanced metabolism of herbicides (Han *et al.* 2016). Multiple resistance was also common in *L. rigidum* populations from Western Australia (Owen *et al.* 2014). However, for Italian ryegrass from Oregon only about 35% of populations had multiple resistance (Rauch *et al.* 2010). Multiple resistance to herbicides was also identified in resistance surveys for *Avena fatua* L. (wild oat) in Canada (Beckie *et al.* 2013) *Raphanus raphanistrum* L. (wild radish) in Western Australia (Owen *et al.* 2015) and for *A. rudis* L. (waterhemp) in Missouri (Schultz *et al.* 2015).

Conclusion

The current extensive survey of *L. rigidum* from fields across south-eastern Australia has demonstrated that herbicide resistance is widespread in this species and increasing in frequency. Despite this, some regions maintain high levels of susceptibility to some herbicides, offering users the advantage of still being able to use a wider range of herbicides. However, the continuing reliance on herbicides for *L. rigidum* control in crops will inevitably lead to selection for additional resistance. This is evident in the current survey, where resistance was identified to prosulfocarb + S-metolachlor and pyroxasulfone, which have been recently introduced (Boutsalis *et al.* 2014). Despite the availability of newer herbicides, reliance solely on herbicides for control of *L. rigidum* is not sustainable and other practices will be required, such as harvest weed seed control (Walsh *et al.* 2017).

References

Agricultural Biotechnology Council of Australia (2022) GM canola growth in Australia. Available at https://www.abca.com.au/wp-content/uploads/2020/07/GMCanolaGrowthinAustralia_Tables_UpdatedJune2020.pdf [Accessed 11 February 2022]

Beckie HJ, Lozinski C, Shirriff S, Brenzil CA (2013) Herbicide-resistant weeds in the Canadian Prairies: 2007 to 2011. *Weed Technology* **27**, 171–183. doi:10.1614/WT-D-12-00095.1

Bonny S (2008) Genetically modified glyphosate-tolerant soybean in the USA: adoption factors, impacts and prospects. A review. *Agronomy for Sustainable Development* **28**, 21–32. doi:10.1051/agro:2007044

Boutsalis P, Broster JC (2006) Herbicide resistance testing of *Lolium rigidum* by commercial institutions. In 'Proceedings of the 15th Australian weeds conference'. Adelaide, SA. (Eds C Preston, JH Watts, ND Crossman) pp. 448–490 (Weed Management Society of South Australia: Adelaide, SA)

Boutsalis P, Preston C, Broster JC (2006) Management of trifluralin resistance in annual ryegrass (*Lolium rigidum* Gaudin) in southern Australia. In 'Proceedings of the 15th Australian Weeds Conference'. Adelaide, SA. (Eds C Preston, JH Watts, ND Crossman) pp. 507–510. (Weed Management Society of South Australia: Adelaide, SA)

Boutsalis P, Gill GS, Preston C (2012) Incidence of herbicide resistance in rigid ryegrass (*Lolium rigidum*) across southeastern Australia. *Weed Technology* **26**, 391–398. doi:10.1614/WT-D-11-00150.1

Boutsalis P, Gill GS, Preston C (2014) Control of rigid ryegrass in Australian wheat production with pyroxasulfone. *Weed Technology* **28**, 332–339. doi:10.1614/WT-D-13-00094.1

Broster JC, Koetz EA, Wu H (2011) Herbicide resistance levels in annual ryegrass (*Lolium rigidum* Gaud.) in southern New South Wales. *Plant Protection Quarterly* **26**, 22–28.

Broster JC, Koetz, EA, Wu H (2012) Herbicide resistance frequencies in ryegrass (*Lolium* spp.) and other grass species in Tasmania. *Plant Protection Quarterly.* **27**, 36–42.

Broster JC, Koetz EA, Wu H (2013) Herbicide resistance levels in annual ryegrass (*Lolium rigidum* Gaud.) and wild oat (*Avena* spp.) in southwestern New South Wales. *Plant Protection Quarterly* **28**, 126–132.

Broster JC, Pratley JE, Ip RHL, Ang L, Seng KP (2019*a*) A quarter of a century of monitoring herbicide resistance in *Lolium rigidum* in Australia. *Crop & Pasture Science* **70**, 283–293. doi:10.1071/CP18584

Broster JC, Pratley JE, Ip RHL, Ang L, Seng KP (2019*b*) Cropping practices influence incidence of herbicide resistance in annual ryegrass (*Lolium rigidum*) in Australia. *Crop & Pasture Science* **70**, 77–84. doi:10.1071/CP18355

Brunton DJ, Boutsalis P, Gill G, Preston C (2018) Resistance to multiple PRE herbicides in a field-evolved rigid ryegrass (*Lolium rigidum*) population. *Weed Science* **66**, 581–585. doi:10.1017/wsc.2018.31

Brunton DJ, Boutsalis P, Gill G, Preston C (2019) Resistance to very-long-chain fatty-acid (VLCFA)-inhibiting herbicides in multiple field-selected rigid ryegrass (*Lolium rigidum*) populations. *Weed Science* **67**, 267–272. doi:10.1017/wsc.2018.93

Duke SO, Powles SB (2008) Glyphosate: a once-in-a-century herbicide. *Pest Management Science* **64**, 319–325. doi:10.1002/ps.1518

Gill G (1996) Ecology of annual ryegrass. *Plant Protection Quarterly* **11**, 195–198.

Hall LM, Beckie HJ, Low R, Shirriff SW, Blackshaw RE, Kimmel N, Neeser C (2014) Survey of glyphosate-resistant kochia (*Kochia scoparia* L. Schrad.) in Alberta. *Canadian Journal of Plant Science* **94**, 127–130. doi:10.4141/cjps2013-204

Han H, Yu Q, Owen MJ, Cawthray GR, Powles SB (2016) Widespread occurrence of both metabolic and target-site herbicide resistance mechanisms in *Lolium rigidum* populations. *Pest Management Science* **72**, 255–263. doi:10.1002/ps.3995

Heap I, Duke SO (2018) Overview of glyphosate-resistant weeds worldwide. *Pest Management Science* **74**, 1040–1049. doi:10.1002/ps.4760

Lemerle D, Verbeek B, Coombes N (1995) Losses in grain yield of winter crops from *Lolium rigidum* competition depend on crop species, cultivar and season. *Weed Research* **35**, 503–509. doi:10.1111/j.1365-3180.1995.tb01648.x

Llewellyn RS, Lindner RK, Pannell DJ, Powles SB (2002) Resistance and the herbicide resource: perceptions of Western Australian grain growers. *Crop Protection* **21**, 1067–1075. doi:10.1016/S0261-2194(02)00091-1

Llewellyn RS, D'Emden FH, Kuehne G (2012) Extensive use of no-tillage in grain growing regions of Australia. *Field Crops Research* **132**, 204–212. doi:10.1016/j.fcr.2012.03.013

Llewellyn R, Ronning D, Clarke M, Mayfield A, Walker S, Ouzman J (2016) Impact of Weeds on Australian Grain Production: the cost of weeds to Australian grain growers and the adoption of weed management and tillage practices. Grains Research and Development Corporation, Canberra, Australia.

McDonald JH (2014) 'Handbook of biological statistics.' 3rd edn. (Sparky House Publishing: Baltimore, MD, USA)

O'Donovan JT, Harker KN, Clayton GW, Blackshaw RE (2006) Comparison of a glyphosate-resistant Canola (*Brassica napus* L.) system with traditional herbicide regimes. *Weed Technology* **20**, 494–501. doi:10.1614/WT-05-092R.1

Owen MJ, Powles SB (2018) Current levels of herbicide resistance in key weed species in the WA grain belt. GRDC Update Papers 26 February 2018. Grains Research and Development Corporation, Canberra, ACT. Available at https://grdc.com.au/resources-and-publications/grdc-update-papers/tab-content/grdc-update-papers/2018/02/current-levels-of-herbicide-resistance-in-key-weed-species.

Owen MJ, Martinez NJ, Powles SB (2014) Multiple herbicide-resistant *Lolium rigidum* (annual ryegrass) now dominates across the Western Australian grain belt. *Weed Research* **54**, 314–324. doi:10.1111/wre.12068

Owen MJ, Martinez NJ, Powles SB (2015) Multiple herbicide-resistant wild radish (*Raphanus raphanistrum*) populations dominate Western Australian cropping fields. *Crop & Pasture Science* **66**, 1079–1085. doi:10.1071/CP15063

Powles SB, Lorraine-Colwill DF, Dellow JJ, Preston C (1998) Evolved resistance to glyphosate in rigid ryegrass (*Lolium rigidum*) in Australia. *Weed Science* **46**, 604–607. doi:10.1017/S0043174500091165

Pratley J, Urwin N, Stanton R, Baines P, Broster J, Cullis K, Schafer D, Bohn J, Kruger R (1999) Resistance to glyphosate in *Lolium rigidum*. I. Bioevaluation. *Weed Science* **47**, 405–411. doi:10.1017/S0043174500091992

Rauch TA, Thill DC, Gersdorf SA, Price WJ (2010) Widespread occurrence of herbicide-resistant Italian ryegrass (*Lolium multiflorum*) in Northern Idaho and Eastern Washington. *Weed Technology* **24**, 281–288. doi:10.1614/WT-D-09-00059.1

Reeves TG (1976) Effect of annual ryegrass (*Lolium rigidum* Gaud.) on yield of wheat. *Weed Research* **16**, 57–63. doi:10.1111/j.1365-3180.1976.tb00379.x

Saini RK, Malone J, Preston C, Gill G (2015*a*) Target enzyme-based resistance to clethodim in *Lolium rigidum* populations in Australia. *Weed Science* **63**, 946–953. doi:10.1614/WS-D-14-00176.1

Saini RK, Kleemann SGL, Preston C, Gill GS (2015*b*) Alternative herbicides for the management of clethodim-resistant rigid ryegrass (*Lolium rigidum*) in faba bean (*Vicia faba* L.) in Southern Australia. *Weed Technology* **29**, 578–586. doi:10.1614/WT-D-14-00143.1

Schultz JL, Chatham LA, Riggins CW, Tranel PJ, Bradley, KW (2015) Distribution of herbicide resistances and molecular mechanisms conferring resistance in Missouri waterhemp (*Amaranthus rudis* Sauer) populations. *Weed Science* **63**, 336–345. doi:10.1614/WS-D-14-00102.1

Tranel PJ, Wright TR (2002) Resistance of weeds to ALS-inhibiting herbicides: what have we learned? *Weed Science* **50**, 700–712. doi:10.1614/0043-1745(2002)050[0700:RROWTA]2.0.CO;2

Walsh MJ, Aves C, Powles SB (2017) Harvest weed seed control systems are similarly effective on rigid ryegrass. *Weed Technology* **31**, 178–183. doi:10.1017/wet.2017.6

Yu Q, Powles SB (2014) Resistance to AHAS inhibitor herbicides: current understanding. *Pest Management Science* **70**, 1340–1350. doi:10.1002/ps.3710

Yu Q, Collavo A, Zheng MQ, Owen M, Sattin M, Powles SB (2007) Diversity of acetyl-coenzyme A carboxylase mutations in resistant *Lolium* populations: evaluation using clethodim. *Plant Physiology* **145**, 547–558. doi:10.1104/pp.107.105262

Data availability. The data that support this study are available in the article and upon request to the corresponding author.

Conflicts of interest. Christopher Preston is an Associate Editor of Crop & Pasture Science, but was blinded from the peer-review process for this paper.

Declaration of funding. This research was funded by the Grains Research and Development Corporation grant number UCS00020. The GRDC had no involvement in study design, collection, analysis or interpretation of data, or in writing the manuscript.

Acknowledgements. The authors would like to thank Alicia Merriam, David Brunton, Geetha Velappan, Ruwan Lenorage and Allison Chambers and casual staff and students at Charles Sturt University for technical assistance.

Author affiliations

[A]Gulbali Institute for Agriculture Water Environment, Charles Sturt University, Boorooma Street, Wagga Wagga, NSW 2678, Australia.

[B]School of Agriculture, Food & Wine, University of Adelaide, Glen Osmond, SA 5064, Australia.