A quarter of a century of monitoring herbicide resistance in *Lolium rigidum* in Australia

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Charles Sturt University has operated a commercial herbicide resistance testing service since 1991, following a random survey of the South West Slopes region of New South Wales that identified significant incidence of herbicide resistance in annual ryegrass (*Lolium rigidum* Gaud.). Other surveys of cropping regions of southern Australia conducted at that time also found a significant incidence of resistance. In the subsequent 25-year period, the testing service has received samples from the majority of the southern Australian cropping belt. Overall, 80% of samples tested were resistant to acetyl-CoA carboxylase (ACCase) inhibiting aryloxyphenoxypropionate and phenylpyrazole herbicides, 56% to acetolactate synthase (ALS) inhibiting herbicides, and 24% to ACCase-inhibiting cyclohexanedione herbicides. The incidences of resistance to inhibitors of photosynthesis at PSII, tubulin-formation inhibitors, and 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase inhibiting herbicides have remained <10% of samples tested. The relationships between many herbicide groups and subgroups are discussed, as is the variability in resistance incidence and the forms of cross or multiple resistance for each state. This paper builds on an earlier publication of 14 years of testing history. At >5000 samples, the size and geographical spread of this dataset allows for valuable analyses of the relationships present in herbicide-resistant populations of annual ryegrass.

Additional keywords: cross-resistance, glyphosate, mode of action.

Introduction

In terms of the evolution of herbicide resistance, annual ryegrass (*Lolium rigidum* Gaud.) is one of the most important species worldwide, and the most important in Australia (Powles *et al.* 1996; Heap 2014). Llewellyn *et al.* (2016) estimated that, in Australian winter cereals, herbicide-resistant annual ryegrass added over AU$100 million in extra herbicide costs alone, plus the cost of additional integrated weed-management practices. The first case of herbicide resistance in annual ryegrass in Australia was reported by Heap and Knight (1982) for the herbicide diclofop-methyl (CropLife Australia Mode of Action (MOA) Group A: acetyl-CoA carboxylase (ACCase) inhibitors, chemical family aryloxyphenoxypropionates or 'fops'). Since then, resistant biotypes of annual ryegrass have been recorded for most of the range of selective herbicides used for its control in Australia (Powles and Matthews 1992; Broster and Pratley 2006). The incidence of resistance has increased to the extent that populations of annual ryegrass susceptible to all selective herbicides are a rarity in many regions

across the southern cropping region of Australia (Owen *et al.* 2007; Broster *et al.* 2011, 2013; Boutsalis *et al.* 2012).

Surveys conducted in southern New South Wales, Victoria and South Australia in the early 1990s reported that diclofop-methyl-resistant populations were present in 13%, 7% and 40% of surveyed fields, respectively (Pratley *et al.* 1993; Henskens *et al.* 1996; Nietschke *et al.* 1996). When other herbicide MOA groups or chemical families were screened in New South Wales (sethoxydim, Group A, chemical family cyclohexanediones or 'dims'; chlorsulfuron, Group B, acetolactate synthase (ALS) inhibitors, chemical family sulfonylureas; simazine, Group C, photosystem II (PSII) inhibitors, chemical family triazines; and trifluralin, Group D, inhibitors of microtubule assembly, chemical family dinitroanilines) and in Victoria (sethoxydim; chlorsulfuron; and metribuzin, Group C, chemical family triazinones), 30% of the fields in New South Wales and 35% in Victoria recorded ryegrass resistance to one or more selective herbicides (Pratley *et al.* 1995; Henskens *et al.* 1996).

More recent surveys have shown that, across a major proportion of the southern cropping zone, biotypes of annual ryegrass resistant to either Group A fop or Group B herbicides are more prevalent than susceptible biotypes. Surveys in southern NSW reported that 81% (Broster *et al.* 2011) and 56% (Broster *et al.* 2013) of populations were resistant to diclofop-methyl, and a 2003 survey in Western Australia found that 96% of populations contained ryegrass resistant to a Group A fop herbicide (Owen *et al.* 2014). Several regions in South Australia and Victoria recorded >60% of populations containing ryegrass resistant to Group A fops (Boutsalis *et al.* 2012). In both NSW surveys, in Western Australia, and in all regions surveyed in South Australia and Victoria, the incidence of resistance to Group B herbicides was similar to, or higher than, that for Group A fops. In all but a few South Australian and Victorian regions, Group B resistance occurred in >60% of populations (Broster *et al.* 2011; Boutsalis *et al.* 2012; Broster *et al.* 2013; Owen *et al.* 2014). Despite the widespread high incidence of resistance to both of these herbicide groups, some agronomic regions of these states and across Tasmania still have lower incidences of resistance to these and other herbicide groups used to control annual ryegrass (Broster *et al.* 2011, 2012, 2013; Boutsalis *et al.* 2012; Owen *et al.* 2014).

Charles Sturt University has operated a commercial herbicide-resistance testing service since 1991 with the results from these tests used by farmers both as an aid in developing management strategies to minimise herbicide-resistance evolution and to manage populations on their properties already identified as resistant. The outcomes from the first 14 years of testing (1991–2004) are reported in Broster and Pratley (2006). This paper evaluates the trends from the testing service over a further 11 years, representing a quarter of a century in total.

Materials and methods

The methods used for preparing, growing and testing samples remain mainly as described in Broster and Pratley (2006), with some minor changes made over time. In brief, annual ryegrass samples are

usually received around harvest time late in the year and stored at room temperature until testing from February onwards. Immediately before testing, seeds are placed in a cool room at 2–5°C to overcome any dormancy.

Seeds are sown into trays under glasshouse conditions (day, night temperatures: 25°C, 10°C). Pre-emergent treatments are sprayed before sowing and incorporated or watered in as necessary. Herbicides are applied at the recommended stage of growth by using an automated laboratory-sized cabinet sprayer with a moving boom. Since 2015, the applied water volume has been 85 L ha^{-1} (previously 83 L ha^{-1}) from two even fan nozzles at 250 kPa (previously 300 kPa).

Herbicides are applied at 0, 0.5, 1.0 and 2.0 times their recommended rate (Table 1) on three replicates of 20 seedlings per replicate. A known susceptible biotype and, where available, a known resistant biotype are included for comparison. Treatments are assessed after 3 weeks. Post-emergent treatments are measured for seedling survival at the recommended rate (>20% survival classified as resistant, 10–20% classified as evolving resistance), whereas pre-emergent treatments are rated visually from 0 (no germination) to 10 (equivalent to control), with classifications of resistance (score >2.5) and evolving resistance (1.5–2.5). Where variability masks the resistance status, the test is repeated once with only the second test results utilised.

Statistical analyses

Because all samples are from fields at the end of each crop season and are subject to a standard testing protocol under controlled conditions, the data have been bulked to identify main effects. For this paper, the 'evolving resistance' and 'resistance' classifications were combined into a single resistant category for data analyses.

Trends in the incidence of herbicide resistance (in terms of proportions) over time (in 5-year blocks) were determined by using the Cochran–Armitage test for trend (Cochran 1954; Armitage 1955). The periods were grouped in 5-year cohorts for two reasons: (*i*) ease of comparison, and (*ii*) to avoid unnecessary inflations in proportions due to a low number of tested samples (e.g. 100% resistance due to only one sample). Differences between two sample proportions were analysed by using Fisher's exact test (Agresti 2002). For more than two samples, multiple pairwise comparisons were performed by using the Marascuilo procedure (Marascuilo and McSweeney 1967). A difference or a trend is considered statistically significant where $P < 0.05$.

Table 1. Herbicides commonly evaluated on annual ryegrass samples by the Charles Sturt University commercial herbicide-resistance testing service, and the commercially recommended rates of application of each active ingredient (a.i.)

Herbicides are grouped according to Mode of Action (MOA) classification systems: Australian (CropLife Australia: https://www.croplife.org.au/wp-content/uploads/2017/06/2018-Herbicide-MOA-Table.pdf); WSSA, Weed Science Society of America; HRAC, Herbicide Resistance Action Committee. Herbicides presented have a minimum of 30 samples tested; some other herbicides were evaluated in this study

MOA classification system			Chemical family	Chemical name	Recommended rate
Australian	WSSA	HRAC			(g a.i. ha^{-1})
A	1	A	Aryloxyphenoxypropionates ('fops')	diclofop-methyl	375
				haloxyfop-R	39
			Cyclohexanediones ('dims')	clethodim[A]	60 (120 since 2013)
				sethoxydim	72
				tralkoxydim	200
				butroxydim	45
			Phenylpyrazoles ('dens')	pinoxaden	30
B	2	B	Sulfonylureas	chlorsulfuron	15
				triasulfuron	26
			Imidazolinones	imazapic/imazapyr	28 (2000–09)
				imazamox/imazapyr[B]	36 (since 2010)
C	5	C1	Triazines	simazine	1200
D	3	K1	Dinitroanilines	trifluralin	800
			Benzamides	propyzamide	750
J + K	N + K3	8 + 15	Thiocarbamates + Chloroacetamides	prosulfosarb + S-metolachlor	2000 + 300
K	K3	15	Isoxazolines	pyroxasulfone	100.3
L	22	D	Bipyridyls	paraquat	300
M	9	G	Glycines	glyphosate	576

[A]Recommended rate changed on label.

[B]Change of chemical in recommended commercial product for Clearfield varieties.

Sample cohorts

Since 1991, samples have been received as described on a fee-for-service basis. Farmers or their advisers request tests to confirm suspected resistance or to search for alternative herbicides for known resistant samples. Samples have also been provided as part of ongoing management trials conducted by commercial agronomists or government departments. Reseller chains have also requested individual herbicides different from the standard tests when arranging for testing of a large number of samples from their member clients. Additionally, between 2001 and 2003, samples were provided through a program called WeedSense, sponsored by Dow AgroSciences Australia, with any differences between these samples and the other samples reported in Broster and Pratley (2006). In the present paper, these results for the different cohort sources have not been separated in the analyses.

Results

Source of samples

For the period 1991–2015, 5308 ryegrass samples were received, generating 26 517 individual tests under the protocols described. This includes an additional 2217 samples (42%) and 11 552 tests (44%) since the previous report (Broster and Pratley 2006).

Samples have been received from the winter cropping zone of southern Australia, comprising five states, with 1952 (771 since 2005) from New South Wales, 1736 (1200 since 2005) from Western Australia, 914 (192 since 2005) from Victoria, 677 (25 since 2005) from South Australia, and 29 (all since 2005) from Tasmania. The samples emanate from 410 of the 600 postcodes in that zone, including an extra 80 postcodes since 2004, and thus are comprehensively representative of annual ryegrass in Australian winter crops. Although it is recognised that the postcodes provided may represent the location of the submitter rather than the field, the discrepancy would be slight and has not been considered in this analysis.

Herbicides screened

The number of herbicides tested per sample ranged from one to ten, with the majority of samples tested to five (50.7%) or six (31.4%) herbicides. In total, 524 samples (9.9%) were tested to two or more herbicides in one herbicide MOA group. Another 99 (1.9%) were tested to two herbicide MOA groups, in which at least two herbicides were tested.

Overall incidence of resistance

Of the 5308 samples tested, 1018 (19%) were susceptible to all tested herbicides. However, many of these samples were not tested to particular herbicides groups, most commonly the higher risk groups (Group A fops and Group B). For these samples, it is assumed the client held the view that the sample was most likely resistant to those groups and so wanted to investigate options for herbicide susceptibility.

Overall resistance was highest for Group A fop herbicides (81%), pinoxaden (Group A, chemical family phenylpyrazoles or ‘dens’) (81%) and Group B (56%). Although common, resistance to Group A dim herbicides was relatively low (24%), whereas resistances to Group D dinitroanilines (7%), Group C triazines (1%) and glyphosate (Group M (glycines), 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase inhibitors) (4%) were much lower.

Several other herbicide groups or subgroups have also been tested for incidence of resistance. Three samples (of 382 tested) tested resistant to paraquat or paraquat + diquat (Group L (bipyridyls), PSI inhibitors). No samples have tested positive for resistance to propyzamide (Group D, chemical family benzamides; 286 tested), prosulfocarb + metolachlor (Group J, lipid synthesis inhibitors (non-ACCase inhibitors), chemical family thiocarbamates + Group K, very long-chain fatty acid (VLCFA)

inhibitors, chemical family chloroacetamides; 132 tested) or to pyroxasulfone (Group K, chemical family isoxazolines; 36 tested).

The extent of resistance has increased ($P < 0.001$) for three of the major herbicide groups screened (Group A fops, Groups B and M) since the testing service began. However, only for the 2001–05 period was there a difference between time cohorts for the Group A fop herbicides. In the three 5-year cohorts before 2005 there was no difference in the extent of resistance to Group B, and although there is no difference between the two cohorts since 2005, both have a significantly higher incidence of resistance than occurred before 2005. Since the first testing for Group M resistance in 1996 the extent of resistance has increased; however, only in the 2011–15 period was this significantly higher than for 1996–2000 (Table 2).

Table 2. Proportions of resistant samples of annual ryegrass and number of samples tested for each herbicide MOA group or subgroup over time

Within rows, values followed by the same letter are not significantly different ($P > 0.05$). Last column reports the upward or downward trend of proportions, with the associated *P*-value for each herbicide group (in parentheses). n.a., not applicable

Herbicide Group		1991–95	1996–2000	2001–05	2006–10	2011–15	Trend
A 'fops'	Proportion	88.8%b	90.4%b	74.5%a	93.2%b	90.8%b	Upward
	No. tested	134	614	2498	512	487	(<0.001)
A 'dims'	Proportion	46.5%c	24.9%ab	21.3%ab	26.6%ab	25.4b	n.s.
	No. tested	86	623	2611	575	1727	
A 'dens'	Proportion	–	–	–	66.7%a	80.2%a	n.a.
	No. tested	0	0	0	33	167	
B	Proportion	46.5%ab	35.1%a	45.1%ab	87.9%c	84.1%c	Upward
	No. tested	114	567	2393	545	656	(<0.001)
C	Proportion	1.8%ab	5.2%b	0.3%a	0.2%a	0.3%a	Downward
	No. tested	57	461	2257	509	1329	(<0.001)
D	Proportion	22.2%bc	12.5%c	7.5%b	9.4%bc	3.6%a	Downward
	No. tested	63	503	2267	523	1373	(<0.001)
M	Proportion	–	1.4%a	0.4%a	10.2%ab	6.1%b	Upward
	No. tested	0	70	1126	49	1188	(<0.001)
	Samples	113	655	2565	570	1405	

Groups C and D have shown decreases ($P < 0.001$) in the extent of resistance, and there has been no significant trend in the extent of resistance to Group A dims (Table 2). For Group D, resistance has dropped significantly from the first time cohort until the last (Table 2).

Broster and Pratley (2006) reported that the only difference between the Charles Sturt University samples and the WeedSense samples (2001–05) was for the Group A fop and Group B herbicides. Excluding the WeedSense samples from the results for these groups from that time cohort increases the incidence of resistance for that period to 82.9% and 53.9%, respectively, but does not change the trends observed for either herbicide group.

Patterns of resistance

In Group A there was a strong correlation with respect to resistance between Group A fops and pinoxaden (Group A dens). However the correlation was weaker between Group A dims and either Group A fops or pinoxaden (Table 3). Whereas differences between Group A fop herbicides were small, significant differences occurred among the Group A dims. The relationship between clethodim and sethoxydim or tralkoxydim was weaker than that between sethoxydim or tralkoxydim and the Group A fop herbicides, and between clethodim and butroxydim (Table 3). Overall, the incidence of resistance to clethodim (4763 tests, 17% resistant) was similar to that of butroxydim (151 tests, 18% resistant), with both much lower than either sethoxydim (190 tests, 49% resistant) or tralkoxydim (435 tests, 81% resistant). In total, 62 samples were tested to a herbicide product containing both Group A fop and dim chemicals (mostly fluazifop + butroxydim), with 47% of these samples classed as resistant.

Table 3. Relationships between various herbicides or subgroups (chemical families) within Groups A and B with respect to herbicide-resistance status in annual ryegrass

	No. of samples	Same resistance status (%)
Group A		
'Fops'–'fops' (all herbicides)	42	95
'Fops'–'dims' (all herbicides)	3982	39
'Fops'–clethodim	3722	35
'Fops'–sethoxydim	184	68
'Fops'–tralkoxydim	215	80
Clethodim–sethoxydim	30	50
Clethodim–tralkoxydim	319	31
Clethodim–butroxydim	151	81
Pinoxaden ('dens')–'fops'	60	100
Pinoxaden–clethodim	151	34
Group B		
Chlorsulfuron–triasulfuron	38	74
Sulfonylureas–imidazolinones	76	70

Differences between states

Statistically similar incidences of resistance to Group A fop and dim herbicides occurred in New South Wales (85% and 28%, respectively) and Victoria (83% and 24%), with Western Australia (79% and 22%) showing significantly lower resistance than New South Wales but no difference from Victoria (Table 4). South Australia had lower resistance incidence to both subgroups (69% and 14%) than the other states (Table 4).

Western Australia (74%) had the highest proportion of Group B resistance, significantly higher than all other states, with New South Wales (61%) also showing higher incidence of Group B resistance than both Victoria (37%) and South Australia (32%), which were statistically similar. As was the case from 1991 to 2004 (Broster and Pratley 2006), the states with the highest Group B resistance, Western Australia and New South Wales, had the lowest incidence of Group D resistance

(3% and 5%, respectively), significantly lower than both Victoria (14%) and South Australia (15%) (Table 4). Group C resistance was <1.5% in all states. Group M resistance was highest in New South Wales and Western Australia (5% and 3%, respectively), with none detected in South Australia (Table 4).

Table 4. Proportions of resistant annual ryegrass samples and number of samples tested (in parentheses) from different states over the entire period (1991–2015) for selected herbicide MOA groups and subgroups

Within rows, values followed by the same letter are not significantly different ($P > 0.05$). Data from Tasmania were excluded owing to the low number of samples tested

	NSW	SA	Vic.	WA
A 'fops'	85.2%c	69.4%a	82.8%bc	79.2%b
	(1628)	(653)	(856)	(997)
A 'dims'	28.3%c	14.1%a	23.9%bc	22.4%b
	(2027)	(695)	(869)	(1931)
B	61.2%b	32.0%a	37.4%a	73.8%c
	(1723)	(640)	(767)	(988)
C	0.9%ab	0.5%ab	1.5%b	0.2%a
	(1541)	(582)	(742)	(1628)
D	4.6%a	15.4%b	14.2%b	3.3%a
	(1625)	(597)	(745)	(1911)
M	5.1%b	0.0%a	1.0%a	4.5%b
	(681)	(379)	(299)	(1022)

There was a significant difference in the incidence of resistance to the different Group A dim herbicides, with clethodim and butroxydim resistance significantly lower than sethoxydim and tralkoxydim resistance for all states (and over all samples) (Table 5). There was also a difference within Group B for incidence of resistance recorded to the sulfonylurea and imidazolinone herbicides. Significantly more samples were resistant overall, and in New South Wales and South Australia, to the imidazolinone herbicides than to the sulfonylureas. Differences were not significant for Victoria or Western Australian (Table 5).

Cross-resistance and multiple resistance

Of the 5308 samples received, 3390 were screened to a herbicide from the five standard selective herbicide groups/subgroups (A fops and dims, B, C, D). Only 311 (9%) of these samples were susceptible to all tested herbicides, and 33% of samples (1122) were resistant to only one herbicide group, 73% of which (24% of overall samples) were resistant to Group A fops and 23% (7% overall) to Group B. Of the 1464 samples (43%) resistant to two herbicide groups/subgroups, 75% (32% overall) were resistant to Group A fops and Group B, 18% (8% overall) to Group A fops and dims, and 5% (2% overall) to Group A fops and Group D (Table 6). Of the 455 samples (13%) resistant to three herbicide groups/subgroups, the majority were resistant to Group A fops and dims and Group B (77%, 10% overall), with 17% (2% overall) resistant to Group A fops and Groups B and D, and 5%

(1% overall) to Group A fops and dims and Group D. There were 38 samples (1%) resistant to four herbicide groups/subgroups. All of these samples were resistant to Group A fops and Group B; there were three combinations for the other two herbicides, but for most samples, the additional resistances were to Group A dims and Group D (Table 6).

Table 5. Proportions of annual ryegrass samples resistant and number of samples tested (in parentheses), and significance of difference between different Group A 'dim' herbicides or between Group B subgroups across states

Data from Tasmania are not presented owing to the low number of samples tested, but are in included the Australian total; n.s., not significant ($P > 0.05$)

	Dims			Group B subgroups (chemical families)		
	Clethodim and butroxydim	Sethoxydim and tralkoxydim	*P*-value	Sulfonylureas	Imidazolinones	*P*-value
NSW	18.4 (1682)	82.5 (308)	<0.001	60.7 (1585)	84.4 (77)	<0.001
SA	11.5 (652)	53.5 (43)	<0.001	29.6 (591)	63.0 (46)	<0.001
Vic	18.3 (774)	71.1 (90)	<0.001	37.4 (736)	43.5 (23)	n.s.
WA	19.6 (1625)	55.9 (186)	<0.001	73.8 (940)	79.2 (24)	n.s.
Australia	17.9 (4762)	71.0 (630)	<0.001	55.0 (3881)	71.5 (172)	<0.001

Of the 3390 samples screened to herbicides from the five standard selective herbicide groups/subgroups, 1278 were supplied from New South Wales, 551 from South Australia, 26 from Tasmania, 681 from Victoria, and 854 from Western Australia. South Australia had the highest proportion (19%) of samples susceptible to all herbicides, being significantly higher than the other states (Tasmania was excluded from this analysis due to low sample numbers).

Samples from South Australia and Victoria (both 42%) were more likely to be resistant to only one herbicide than those from New South Wales (30%) and Western Australia (26%) (Table 6). Samples from New South Wales, South Australia and Victoria resistant to only one herbicide group were more likely to be resistant to Group A fops than Group B, whereas resistances to Group A fops (12%) and Group B (13%) were similar in Western Australia (Table 6).

Approximately half of the samples from New South Wales and Western Australia were resistant to two groups, significantly higher than the incidence recorded for South Australia (28%) and Victoria (35%) (Table 6). For all states, when samples were resistant to only two groups, these were most likely to be Group A fops and Group B, followed by Group A fops and dims, and then Group A fops and Group D. Higher proportions were resistant to only Group A fops and Group B in New South Wales and Western Australia (79% and 85%, respectively) than in South Australia and Victoria (53% and 56% respectively). Conversely, resistance to Group A fops and Group D only was more common among samples from South Australia and Victoria despite the lower proportion of samples resistant to only two groups from these states (Table 6).

Western Australia had the highest proportion of samples resistant to three groups (18%), significantly higher than both Victoria (12%) and South Australia (8%) but not New South Wales (13%). Samples from New South Wales and Western Australia were more likely to contain a Group B as one of the three herbicides, whereas those from Victoria and South Australia were more likely to show resistance to Group D (Table 6).

Table 6. Proportions of resistant annual ryegrass samples for selected (multiple) herbicide MOA groups and subgroups in different states over the entire period (1991–2015)

Within rows, values followed by the same letter are not significantly different ($P > 0.05$). Last column reports the values combining all states including Tasmania

	NSW	SA	Vic	WA	Australia
No. of samples tested	1278	551	681	854	3390
0 group	7.3%a	19.4%b	9.3%a	5.6%a	9.2%
1 group only	30.0%a	42.3%b	41.7%b	25.8%a	33.1%
A ‘fops’	22.7%b	33.4%c	35.5%c	12.1%a	24.2%
B	6.7%b	6.2%ab	3.4%a	13.1%c	7.6%
2 groups only	48.6%b	28.1%a	35.4%a	50.6%b	43.2%
A ‘fops’ and A ‘dims’	8.5%a	7.3%a	8.4%a	7.0%a	7.8%
A ‘fops’ and B	38.6%b	14.9%a	19.8%a	43.1%b	32.2%
A ‘fops’ and D	0.8%a	4.4%b	6.3%b	0.1%a	2.3%
3 groups only	13.3%cd	8.2%ab	12.0%bc	17.6%d	13.4%
A ‘fops’, A ‘dims’ and B	11.6%c	2.9%a	6.9%b	15.8%c	10.4%
A ‘fops’, A ‘dims’ and D	0.1%ab	1.5%bc	1.5%c	0.0%a	0.6%
A ‘fops’, B and D	1.4%a	3.8%a	3.1%a	1.8%a	2.3%
4 groups only	0.9%a	2.0%a	1.6%a	0.5%a	1.1%
A ‘fops’, A ‘dims’, B and D	0.5%a	1.5%a	1.6%a	0.5%a	0.9%

Glyphosate testing and resistance

Of the 5308 samples received, 2377 (45%) were tested for resistance to glyphosate, the first being tested in 1995–96 and found to be resistant (Pratley *et al.* 1996; Pratley *et al.* 1999). Forty-five samples (33%) were tested for resistance to glyphosate in 1997, after which testing decreased to <2% until increasing in 2002 (69% screened) and 2003 (82% screened) when glyphosate was a standard herbicide for WeedSense. Testing for glyphosate resistance then decreased to 5–20% of samples until 2011, at which time 55% of samples were screened. Since then the proportion of samples tested for resistance to glyphosate has been >70%, with 96% of samples tested in 2015.

Of the 84 samples testing positive for glyphosate resistance, 46 have been from Western Australia, 35 from New South Wales, and three from Victoria. Many of these have also been tested against other herbicides. For most of the selective herbicides or herbicide combinations, the frequencies of resistance were significantly higher for the glyphosate-resistant samples than overall (Table 7). Resistance to Group D was less common in the glyphosate-resistant populations, but this reflects the incidence of resistance in the states from where the glyphosate-resistant samples were supplied,

Western Australian and New South Wales samples having lower frequencies of Group D resistance than Victoria and South Australia (Table 7).

Table 7. Incidence of resistances among glyphosate (Group M)-resistant annual ryegrass samples relative to the overall mean for a specific herbicide MOA group/subgroup or combination of groups

n.s., Not significant ($P > 0.05$)

	No. of samples tested	Group M resistant (%)	Overall resistant (%)	Significance
A 'fops'	20	70.0	80.8	n.s.
A 'dims'	72	36.1	23.8	<0.05
A 'dens'	10	100	80.7	<0.05
B	28	75.0	55.5	<0.05
C	70	0	0.7	n.s.
D	70	1.4	7.3	<0.05
A 'fops' and 'dims'	20	35.0	20.9	<0.01
A 'fops' and B	18	50	46.5	n.s.
A 'fops' and D	17	0	6.3	<0.05
A 'fops' and 'dims' and B	18	27.8	12.0	<0.001
A 'fops' and 'dims' and D	17	0	1.4	n.s.
A 'fops', B and D	15	0	3.3	n.s.

The incidences of resistance on a postcode scale can be seen for Group A fops and dims and Groups B, D and M in Figs 1–3. These reflect differences on a more localised scale and are the results of differences in managerial, agronomic or climatic factors (Broster *et al.* 2019). Changes between the two time cohorts can initially be seen for all groups through the increased number of postcodes providing samples.

For the herbicide groups with the higher incidence of resistance (Group A fops and Group B), increases are seen across all states for most postcodes (Fig. 1). This is also apparent for Group A dims and Group D in regions where resistance was more common for Group A fops and Group B before 2004, most noticeably in Western Australia (Figs. 1 and 2). Among the postcodes on the margins of the cropping region, increases are also seen in the higher risk groups (A fops and B) (Fig. 1) but for some postcodes the incidence of resistance has declined in groups with lower overall resistance (A dims and D) (Fig. 2). The incidence of glyphosate resistance, while still relatively low, has increased from occurring in isolated populations before 2004 to being more widespread across the regions by 2015 (Fig. 3).

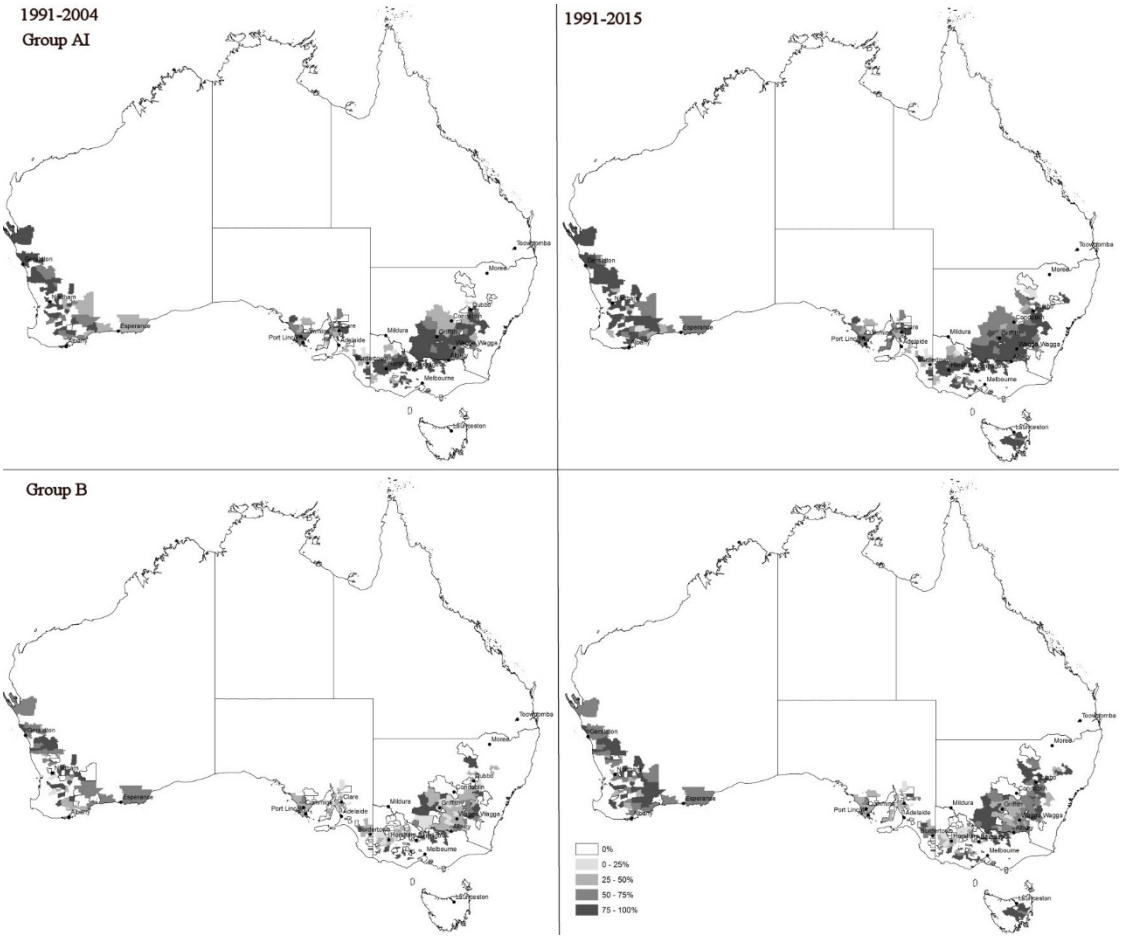


Fig. 1. Incidence of annual ryegrass resistance by postcode from 1991 to 2004 (left) and 1991 to 2015 (right) for Group A 'fops' and Group B herbicides.

Discussion

Herbicide resistance continues to be widespread with the intensity increasing and boundaries spreading. Despite many samples being received for testing from postcodes not previously tested, the incidence of resistance for most herbicides has increased or remained relatively constant. The decline in Group D resistance reported in Table 2 does not represent a decline in resistance to this herbicide but rather a decline in the number of samples received from Victoria and South Australia, the states where resistance to this herbicide group is highest. This suggests that the additional annual herbicide cost of $100 million attributed by Llewellyn *et al.* (2016) is likely to increase, as will the complexity and cost of additional integrated weed-management practices.

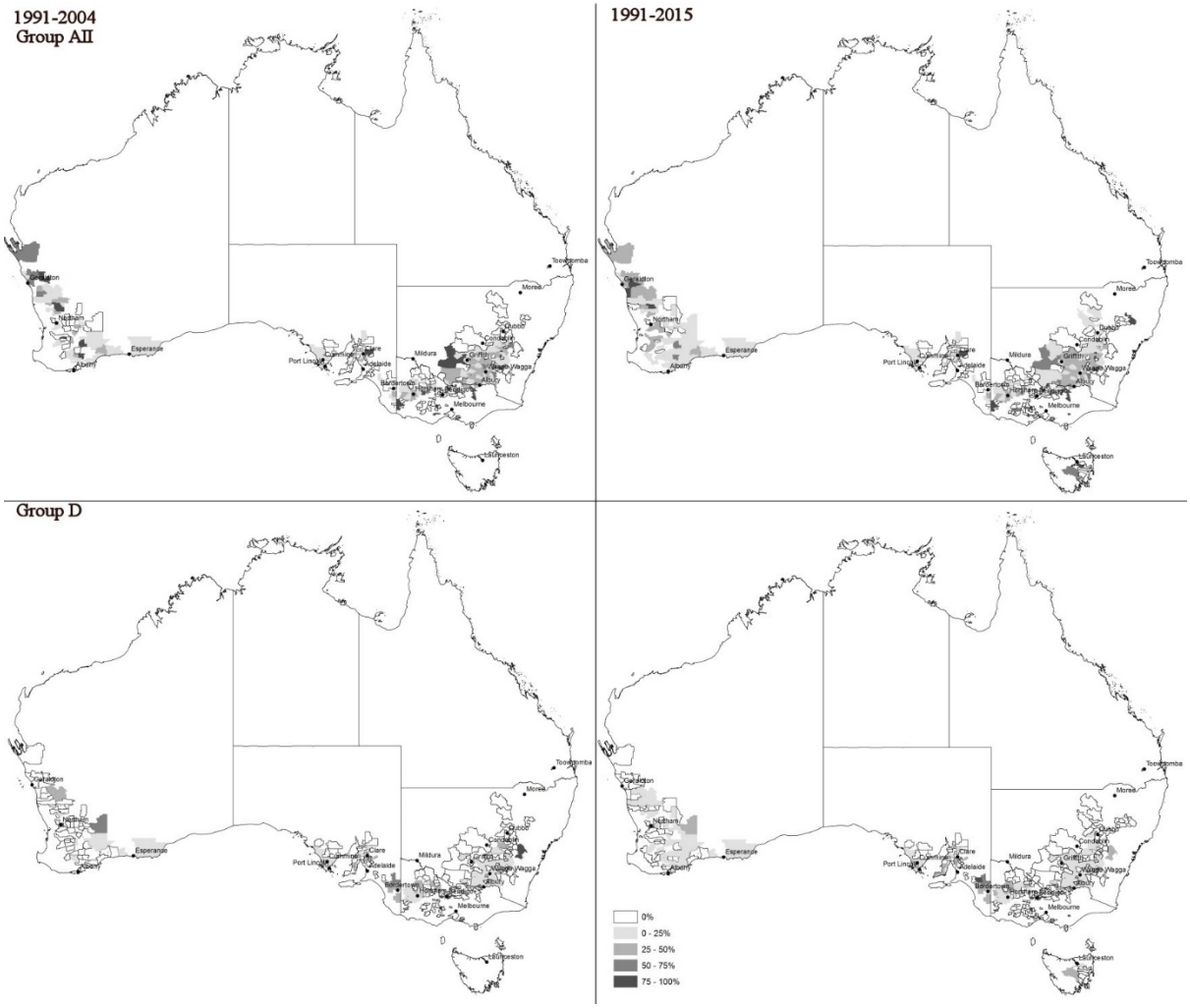


Fig. 2. Incidence of annual ryegrass resistance by postcode from 1991 to 2004 (left) and 1991 to 2015 (right) for Group A 'dims' and Group D herbicides.

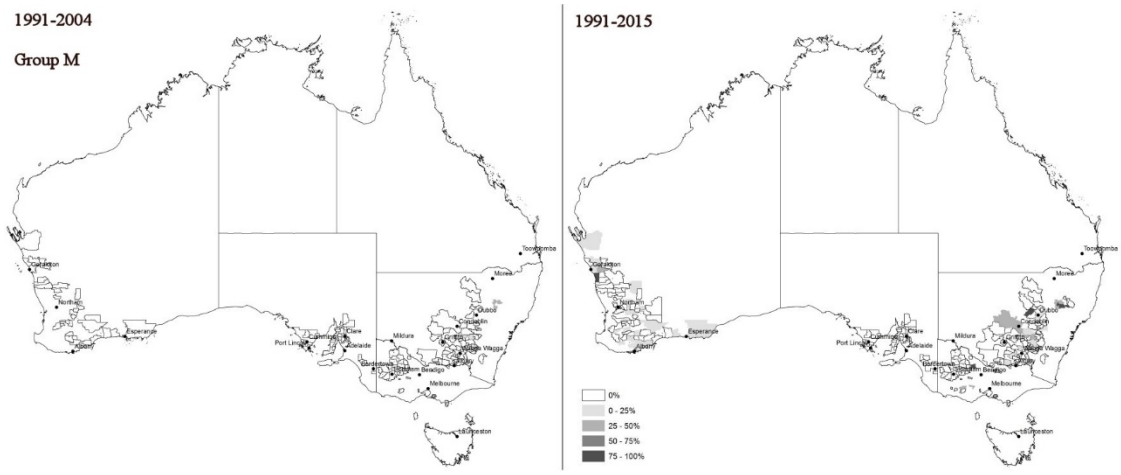


Fig. 3. Incidence of annual ryegrass resistance by postcode from 1991 to 2004 (left) and 1991 to 2015 (right) for Group M herbicides (glyphosate).

Clients require a reason for sending in a sample for resistance screening. It was originally thought that a failure of Group A fops was the trigger for providing a sample. The fact that >80% of samples

are resistant to Group A fop herbicides in most years (and higher percentages if only samples provided at no cost to the farmer through chemical company or reseller promotions are considered) could confirm this hypothesis. With surveys across Australia reporting high levels of Group A fop resistance (Broster *et al.* 2011, 2013; Boutsalis *et al.* 2012; Owen *et al.* 2014), the failure of Group B herbicides seems to have become the trigger for the provision of a resistance test.

The most marked increase in the extent of herbicide resistance across the reported period is for the Group B herbicides. In the previous paper reporting on the testing service, Broster and Pratley (2006) stated that 40% of samples were resistant to Group B herbicides, compared with 77% resistant to Group A fop herbicides. Random surveys conducted, both before (Gill 1993; Pratley *et al.* 1993; Henskens *et al.* 1996; Llewellyn and Powles 2001; Owen *et al.* 2005) and since (Broster *et al.* 2011; Boutsalis *et al.* 2012; Broster *et al.* 2012, 2013; Owen *et al.* 2014) have reported the extent of resistance to Group B to be similar to, or in many cases higher than, that of Group A fop herbicides. Broster and Pratley (2006) speculated that this could occur because the newer Group B herbicides were used as the final control method, similar to the Group A fop herbicides.

Farmers and agronomists accept that many of the older chemistries have lost efficacy (e.g. diclofop-methyl and chlorsulfuron) and they readily seek to confirm the usefulness of new or alternative herbicides, thereby reducing the proportion of samples tested against the older herbicides over the last 5 years (Table 2). Several herbicides that have been chosen as replacements for the standard herbicides have been newly released chemicals, suggesting that the tests were used to confirm alternative herbicides as effective rather than confirming sample resistance. For example, pyroxasulfone (Group K) was first available commercially in Australia for the 2012 season, and the first screening of samples occurred during the 2012 testing period, the seed having been collected from the 2011 crop. The farmer in this case was not prepared to buy the herbicide until a resistance test was done and susceptibility confirmed (D. Crowley, pers. comm.). Similarly, pinoxaden was registered in Australia in May 2006, with the first test conducted in that year (seed collected from 2005 crop). In the case of prosulfocarb + metolachlor, however, it was several years after registration (January 2008) before the first sample was screened in 2013.

With the high incidence of resistance to both Group A fops and Group B, increased selection pressure has been placed on alternative selective herbicides, resulting in increased incidence of resistance to those herbicides. This is apparent in several ways: first, while most clients request the cross-resistance test, the majority of these tests are now customised by not testing Group A fops and Group B as effective herbicide options are sought for known resistant populations; second, the higher number of Western Australian and New South Wales postcodes providing samples that are resistant to trifluralin (Group D) (Fig. 2); and third, in the incidence of resistance to the Group A dim herbicides in many regions, especially in Western Australia. Random surveys in Western Australia also reflect this increase in resistance to Group A dims and Group D herbicides; in the 1998 survey, only one

population was resistant to clethodim (Group A dims) and trifluralin (Group D) was not even tested (Llewellyn and Powles 2001), whereas the 2010 survey found 65% of populations contained plants resistant to clethodim and 27% contained plants resistant to trifluralin (Owen *et al.* 2014).

The small number of samples submitted for testing from Tasmania show an incidence of resistance similar to the mainland states (data not presented), although a random survey (Broster *et al.* 2012) found resistance to be much lower, reflecting the lower intensity and shorter history of chemical use in that state. The presence of herbicide resistance, albeit at a lower incidence, indicates the need for herbicide-resistance best-management practices to be implemented for this region.

The relationships between various herbicide groups and subgroups in the last decade have not altered greatly from that shown by Broster and Pratley (2006). A high correlation relating to resistance incidence exists within Group A fop herbicides, but the relationships within Group A dim herbicides are much weaker. Both sethoxydim and tralkoxydim show better relationships with Group A fops than with clethodim, another Group A dim (Table 3). However, there is a higher correlation between clethodim and butroxydim. The difference between clethodim and both sethoxydim and tralkoxydim is also demonstrated in Table 5 and apparent in the time cohort data in Table 2, and has been recorded in random surveys (Broster *et al.* 2011, 2013; Boutsalis *et al.* 2012; Owen *et al.* 2014). Clethodim has only been used in the testing service since 1995. In the 1991–95 cohort when clethodim comprised 28% of Group A dim tests, 46.5% of samples were resistant to the Group A dim herbicides; since then, 87% of Group A dim tests have been to clethodim and resistance levels have been lower at 21.3–26.6% of samples tested (Table 2). These differences between the Group A dim herbicides are apparent for all states (Table 5).

Differences between the sulfonylurea and imidazolinone subgroups of Group B are also apparent, although not as consistent as for the Group A dim herbicides. The present results are similar to those reported from random surveys; 70% of samples tested to both a sulfonylurea herbicide and an imidazolinone herbicide by the testing service had the same resistance status for both, and the surveys of southern and south-western New South Wales reported that 69% and 78% of samples, respectively, had the same resistance status for both (Broster *et al.* 2011, 2013). The extent of resistance to the imidazolinone herbicides was found to be higher than for the sulfonylureas in the present study. Although this could be because the imidazolinone chemistry was released later in Australia, there are minimal differences in the extent of sulfonylurea herbicide resistance (data not shown) when only samples tested to sulfonylureas since the first imidazolinone test (2000) are included. Rather it is most likely that the imidazolinone tests have been requested to confirm whether this herbicide group is an effective alternative on samples not tested to sulfonylurea herbicides because they are already known to be resistant to that subgroup.

The newest subgroup of Group A herbicides, the phenylpyrazoles (A dens), showed 100% correlation with Group A fop herbicides and a relationship to clethodim similar to the Group A fop herbicides. This is in agreement with Boutsalis *et al.* (2012), who reported a strong relationship between diclofop-methyl, tralkoxydim and pinoxaden in resistance status whereas the relationship of these herbicides with clethodim was much lower. This suggests that, for herbicide resistance in annual ryegrass, the Group A dens could be considered part of the Group A fop herbicides.

Despite the high incidence of resistance to many herbicides, several older herbicides (e.g. triazines and glyphosate) still show low incidence of resistance, and some of the newer chemistries (pyroxasulfone and prosulfocarb + metolachlor) have shown no commercial resistance during the time-frame of this paper. However, resistance to pyroxasulfone has been shown to evolve under experimental conditions if used inappropriately (Busi *et al.* 2012). More recently, a population has been shown to have evolved resistance to prosulfocarb under field conditions (Brunton *et al.* 2018). This confirms the continued opportunity for the development and implementation of appropriate herbicide-resistance management practices to maintain the effectiveness of these and other chemicals for the longer term in Australian farming systems.

In the previous paper, Broster and Pratley (2006) suggested that random benchmarking surveys be conducted on a 5-year basis because testing services tend to be biased towards resistant samples. This is now standard practice enabling the industry to identify trends more readily and develop management and extension programs for the different regions across the Australian winter-cropping zone. Surveys obtain data from regions of less-intense cropping and therefore slower resistance evolution but achieve earlier identification of problems and perhaps identify different management practices. Such surveys also provide a more complete picture without the bias created by a client-focused commercial service. Despite this bias, however, the data from the testing service reported here provide similar findings to the random surveys, as stated by Owen *et al.* (2007) when comparing the findings of the previous paper (Broster and Pratley 2006) with those of a random survey of the Western Australian wheatbelt.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors acknowledge the contributions from the following people in aspects of the work presented in this paper: P Baines, R Graham, A Leys, J Ingrey, A Chambers. The assistance of many students from Charles Sturt University over the 25 years of the resistance testing service is much appreciated. The following herbicide companies contributed chemicals to support the testing service: Bayer CropScience, BASF Australia, CropCare Australasia, Dow AgroSciences, DuPont, Monsanto, Nufarm, Sumitomo Chemicals and Syngenta Crop Protection. This research did not receive any specific funding.

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