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Widespread occurrence of multiple herbicide resistance in Western Australian annual ryegrass (*Lolium rigidum*) populations

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Abstract. In 2003, a random survey was conducted across the Western Australian wheatbelt to establish the frequency and distribution of herbicide resistance in ryegrass populations infesting crop fields. Five hundred cropping fields were visited at crop maturity, and ryegrass seed was collected in 452 of these fields. Subsequently, each crop field population was screened with herbicides of various modes of action that are commonly used for ryegrass control in Australian cropping systems. Most of these ryegrass populations were found to be resistant to the ACCase-inhibitor herbicide diclofop-methyl (68%) and the ALS-inhibitor herbicide sulfometuron (88%). A comparison of resistance levels in the same agronomic zones surveyed 5 years earlier determined that there had been an increase of 20 percentage points in the frequency of resistance over this 5-year period. This survey also determined that the majority (64%) of populations were found to be multiple resistant to both diclofop-methyl and sulfometuron. The distribution patterns of the collected populations indicated that there were higher frequencies of resistant and developing resistance populations occurring in the intensively cropped regions of the wheatbelt, which had greater herbicide selection pressure. Of concern is that 24% and 8% of populations were found to be developing resistance to trifluralin and clethodim, respectively. Currently these herbicides are heavily relied upon for control of ACCase and ALS herbicide resistant ryegrass. Nearly all populations remain susceptible to glyphosate. Ryegrass across the WA wheatbelt now exhibits multiple resistance across many but not all herbicides, posing severe management and sustainability challenges.

Additional keywords: resistance survey, resistance evolution.

Introduction

In Australia, herbicide-resistant weeds have become a major problem in the large temperate-zone, wheat-dominated cropping area known as the wheatbelt. In Western Australia this encompasses a 14 million hectare crop-production region, characterised by large individual farm enterprises (>2000 ha), low rainfall, and infertile, erosion-prone soils. To minimise soil erosion and retain soil moisture, wheatbelt cropping involves minimal soil disturbance (zero or minimum tillage is universal). Cropping with minimal soil disturbance provides agronomic and environmental sustainability advantages, although it removes tillage as a mechanical crop weed control tool. Herbicides are used for crop weed control across the wheatbelt, especially to address the major weed challenge from the competitive annual ryegrass (*Lolium rigidum*). Ryegrass was previously widely grown across the Australian wheatbelt as a pasture plant, is long naturalized, and often present at high densities. Since the 1970s, ryegrass control in crop fields has been achieved with herbicides. However, ryegrass is a highly genetically variable cross-pollinated species and herbicide over-reliance has resulted in the widespread occurrence of herbicide-resistant weed populations. Diclofop-methyl was the first acetyl coenzyme A carboxylase herbicide used in the wheatbelt (from 1978) and similarly, chlorsulfuron was the first acetolactate synthase herbicide used (from 1982). (Hereinafter referred to as ACCase and ALS herbicides.) Since then, various ACCase and ALS herbicides have been extensively used. ACCase and ALS herbicide resistance was evident early in ryegrass in the wheatbelt (Heap and Knight 1982; Christopher *et al*. 1992; Broster and Pratley 2006). Similarly, worldwide, resistance to ALS-inhibiting herbicides is the most common form of weed herbicide resistance, indicating the relative ease with which plants can evolve resistance to these herbicides (reviewed by Saari *et al*. 1994; Tranel and Wright 2002; Heap 2006).

Since the early 1980s, resistant ryegrass populations have exhibited complex herbicide resistance patterns (multiple and cross resistance) across several herbicide chemistries/modes of action (Heap and Knight 1986; Christopher *et al*. 1992; Tardif *et al*. 1993; Burnet *et al*. 1994*a*; Powles *et al*. 1998; Preston and Powles 2002*a*; Broster and Pratley 2006). Multiple resistance exists where there are 2 or more mechanisms of resistance conferring resistance across 2 or more herbicide chemistries (Hall *et al*. 1994). Multiple resistance across several herbicide chemistries/modes of action is well documented in Australian herbicide-resistant ryegrass populations (Heap and

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Knight 1986; Burnet *et al*. 1994*a*; Preston *et al*. 1996; Llewellyn and Powles 2001; Preston and Powles 2002*a*; Broster and Pratley 2006).

For herbicide resistance, as for other pesticide/antibiotic resistance, it is challenging but important to establish the scope of resistance. Few studies have quantified the geographical extent and severity of herbicide resistance across large regions. Canadian field surveys of the major crop weed *Avena fatua*, revealed the extent of resistance to herbicides that inhibit the plastid enzymes ACCase and ALS (Beckie *et al*. 1999, 2004; Legere *et al*. 2000). In Australia, ryegrass in many areas has evolved multiple herbicide resistance (Broster and Pratley 2006), and a 1998 random survey across a 6-million-ha section of the Western Australian (WA) wheat-belt identified very high levels of resistance to commonly used herbicides (Llewellyn and Powles 2001; also see Gill 1995). In the 1998 survey, 191 populations were tested; 46% exhibited resistance to diclofop-methyl and 64% to chlorsulfuron, with 37% exhibiting multiple resistance to both herbicides.

This paper reports the results of a large random survey conducted in 2003, to determine the frequency and distribution of resistance to commonly used herbicides in crop field ryegrass populations collected across the WA wheatbelt. Changes in the extent of herbicide resistance over the 5-year period since the 1998 survey (Llewellyn and Powles 2001) are quantified together with characterisation of common multiple resistance patterns.

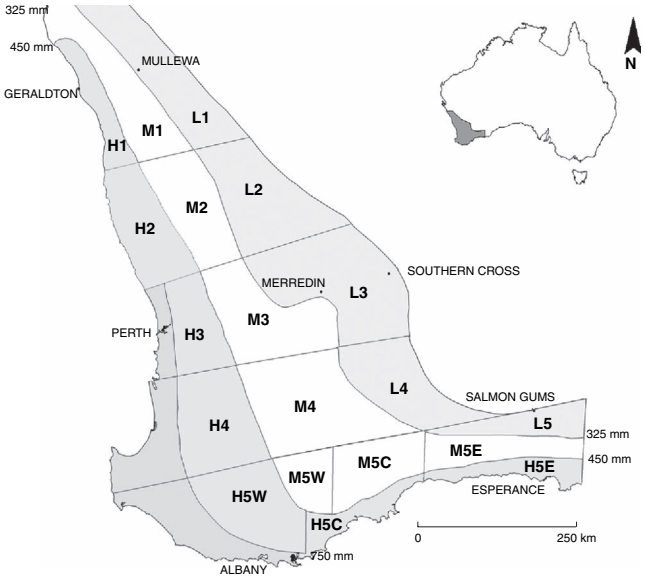

Fig. 1. Map of south-western Western Australia showing the agronomic zones of the wheatbelt where annual ryegrass samples were collected for herbicide resistance testing. Average annual rainfall isohyets are shown. Rainfall regions are shown by H (high, 450–750 mm), M (medium, 325–450 mm), and L (low, <325 mm). Zones are shown by 1 (north), 2 (north-central), 3 (central), 4 (south-central), 5C (south-central), 5E (south-east), and 5W (south-west).

Materials and methods

Crop fields were surveyed over a 5-week period (November and December) just before the 2003 grain harvest. In total, 500 cropping fields were visited across 15 agronomic zones in the WA wheatbelt (Fig. 1). These areas are coded as length of growing-season zones from north to south (1–5) (based on mean daily temperature), and rainfall regions based on annual average rainfall, from east to west. Crop fields were randomly visited at 5-km intervals (geo-referenced) in each zone (interspersed pasture fields were not sampled). As expected, wheat (*Triticum aestivum*) was the dominant crop, comprising 335 of the 500 fields (71%), with the remainder comprising oat (*Avena sativa* L.) (12%), barley (*Hordeum vulgare* L.) (10%), lupin (*Lupinus* spp.) (6%), canola (*Brassica napus* L.) (0.5%), and mixed fodder crops either oat/barley or oat/lupin (1%). Fields were surveyed by 2 people walking in an inverted 'V' 100 m into the crop. Fully mature annual ryegrass spikes were collected and bulked from mature plants in the sampling path. Ryegrass was present in nearly all 500 fields but was only collected from 452 fields (90%) (in 48 fields less than 10 seed spikes were found in the sample path, with insufficient seed to constitute a representative sample). Immediately after collection, the ryegrass seed samples were stored in a non air-conditioned glasshouse over summer (December–March) to break seed dormancy, ensuring maximum germination (Steadman *et al*. 2003). The methods described are similar to those used in the 1998 survey (Llewellyn and Powles 2001).

Resistance testing

During the 2004 growing season (May–September), 50 seeds of each of the 452 ryegrass populations were planted in plastic seedling trays (300 mm by 400 mm by 100 mm) containing potting mix (50% composted pine bark, 25% peat, and 25% river sand). Trays were kept outdoors at the University of Western Australia (UWA) and were watered and fertilised as required. For almost all populations, germination and seedling emergence was high (>90%), ensuring that 40 individual seedlings in each population were screened to each herbicide. Seedlings were treated with herbicide (upper recommended field rates) using a custom-built, dual-nozzle cabinet sprayer delivering herbicide in 100 L/ha water at 210 kPa. Known resistant and susceptible ryegrass populations were used as controls for each herbicide treatment. In all experiments, 100% mortality occurred in the known susceptible population (VLR1), whereas with the known resistant populations there was always very high survival with all herbicides used (data not shown). Herbicide effect was assessed by determining seedling mortality 21 days after herbicide treatment. Ryegrass populations were classed as resistant if 20% or more of the individuals in the population survived the herbicide. Where there was 1–19% survival, the population was classed as developing resistance. Where there was less than 1% survival, the population was classed as susceptible. Due to limited seeds for some populations, not all 452 populations were screened to all herbicides, nor were all herbicide treatments replicated.

ACCase herbicides

When ryegrass seedlings were at the 2-leaf stage, the aryloxyphenoxypropionate herbicide diclofop-methyl was applied at 563 g/ha in 2 passes with adjuvant (0.25% v/v BS1000 [1000 g/L alcohol alkoxylate]; Cropcare Australasia

Pty Ltd). Seedlings that survived diclofop-methyl at 21 days after treatment were cut back to a height of 20 mm, allowed to regrow for 1 week, and then treated with the cyclohexanedione ACCase herbicide sethoxydim (186 g/ha) plus adjuvant (0.25% v/v BS1000). Sethoxydim was applied to diclofop-methyl survivors because previous work shows that although ryegrass can metabolise diclofop-methyl, it cannot metabolise sethoxydim (Tardif and Powles 1994). Therefore resistance to sethoxydim indicates target site resistance.

In experiments with the cyclohexanedione herbicide clethodim, 2-leaf stage ryegrass seedlings were treated with clethodim at 60 g/ha in 2 passes with adjuvant (1% v/v Hasten, 704 g/L esterfied [ethyl-based] canola oil and non-ionic surfactants, Victorian Chemicals, Australia). All ryegrass populations were treated with clethodim.

ALS herbicide

Two to three-leaf stage ryegrass seedlings were treated with the sulfonylurea herbicide sulfometuron (15 g/ha plus adjuvant 0.25% v/v BS1000), using the same procedure as described above. Sulfometuron was chosen to evaluate resistance to ALS herbicides because previous work established that this is not metabolised in ryegrass and therefore indicates the presence of target site resistance (Burnet *et al*. 1994*b*).

Triazine herbicide

Plants were grown in trays (300 mm by 400 mm by 100 mm) containing a soil mix (50% Gingin loam, 40% sand, and 10% wood mulch). Atrazine was applied at 1 kg/ha with an adjuvant (0.1% v/v BS1000) when the plants were at the 1.5-leaf stage, using the same procedure as described above. Any seedlings surviving the first atrazine treatment were cut back to a height of 20 mm, allowed to regrow, and then re-sprayed with atrazine at 1 kg/ha. This procedure was repeated to ensure that the seedlings that germinated after the first spraying received an application of herbicide.

Dinitroaniline herbicide

For the pre-emergent herbicide trifluralin, 50 seeds of each population were planted at a depth of 5.0 mm in seedling trays (300 mm by 400 mm by 100 mm) containing potting mix (50% composted pine bark, 25% peat, and 25% river sand) lightly covered with soil, watered, and left for 2 days to allow the seed to imbibe before herbicide treatment. Trifluralin was applied at 960 g/ha. Immediately after spraying, 10 mm of untreated soil was placed on the soil surface to prevent trifluralin volatilisation. Seedling emergence was recorded 21 days after treatment. Only seedlings that had reached the 2–3 leaf stage, and were of comparable growth stage with that of the known resistant control, were recorded as resistant.

Glycine herbicide

Glyphosate was applied at 540 g/ha to 2–3 leaf stage seedlings in 2 passes using the same procedure as described above for diclofop-methyl. Any plants surviving the treatment after 21 days were cut back to a height of 20 mm, allowed to regrow and then re-sprayed with 810 g/ha glyphosate. Mortality was re-assessed 21 days after the 2nd treatment and the survivors were recorded. This procedure was repeated for glyphosate in a second growing season (2005) for the populations that had plants surviving the first screening.

Statistical analysis

For the ryegrass populations screened for resistance to diclofop-methyl and sulfometuron, a 3×3 chi-square contingency table test was used to identify any relationship between resistance status to both diclofop-methyl and sulfometuron (multiple resistance). To do this, populations were given a score of 2 for each diclofop-methyl and sulfometuron resistant classification and 1 for each diclofop–methyl and sulfometuron developing resistant classification. The non-parametric Wilcoxon ranked sum test (Meddis 1984) was then used to determine the significance of changes in diclofop-methyl and sulfometuron scores (resistance) for the 5-year period between the 1998 and 2003 surveys. Proportional reduction in error measures of association for ordinal variables, gamma and Kendall's tau-b, are included to show the strength and direction of association (-1 to 1) for tests with chi-square $P < 0.2$.

Results and discussion

Resistance to ACCase herbicides

Resistance to the aryloxyphenoypropionate herbicide diclofop-methyl

Diclofop-methyl resistance was common in the ryegrass populations randomly collected from the WA wheatbelt. Of the 452 populations treated with diclofop-methyl, 68% (308 populations) displayed some level of resistance (Table 1). As expected, the degree of resistance varied across populations, with 37% classified as resistant (>20% survival but many with high survival). A further 31% of populations were classified

Table 1. The number and (percentage) of resistant ryegrass populations in each category for each herbicide
Zero indicates fully susceptible populations, 1–19% survival results in classification as populations developing resistance, and >20% survival results in classification as resistant populations. The total number of resistant populations (TR) and the total number of populations tested (TP) are shown

Category	Diclofop	Sethoxydim[A]	Clethodim	Sulfometuron	Trifluralin	Atrazine	Glyphosate
0	144 (32)	119 (39)	413 (91.5)	53 (12)	343 (76)	420 (99)	429 (99)
1–19	139 (31)	30 (10)	36 (8)	88 (20)	108 (23.8)	5 (1)	3 (1)
>20	169 (37)	57 (51)	2 (0.5)	300 (68)	1 (0.2)	0	0
TR	308 (68)	187 (61)	38 (8.5)	388 (88)	109 (24)	5 (1)	3 (1)
TP	452	306	451	441	452	425	432

[A]Sethoxydim resistance was only tested on the diclofop-resistant populations.

as developing resistance (1–19% survival). Only 32% of populations were diclofop-methyl susceptible. As expected, the frequency of resistance varied between agronomic regions (Table 2). This indicates that diclofop-methyl resistance is now much more common than susceptibility in ryegrass populations across the WA wheatbelt. The primary explanation for this is the different cropping intensity between agronomic zones and, therefore, different diclofop-methyl selection pressure. Regions with intensive cropping programs have had greater diclofop-methyl usage, and consequently there was a higher frequency of resistant populations (and *vice versa*). This pattern was also found in the 1998 survey (Llewellyn and Powles 2001). For example, region H4 has low cropping intensity (less herbicide usage) and an emphasis on livestock production and, understandably, ryegrass in this region had the lowest degree of resistance. In this H4 region, 27% of the populations had resistant ryegrass plants when compared with intensive cropping areas (e.g. H1, H2, M2) where more than 90% of the ryegrass populations had resistant plants (Table 2).

Screening for target site resistance with the cyclohexanedione herbicide sethoxydim

Whole-plant screening with diclofop-methyl, while unequivocally establishing resistance (Table 1), cannot identify the resistance mechanism(s) present in resistant plants. Resistance could be due to reduced sensitivity of ACCase (target site resistance) and/or to enhanced rates of diclofop-methyl metabolism (non-target site resistance). Previous work has established that different ACCase mutations (Tardif and Powles 1994; Tardif *et al*. 1996; Zhang and Powles 2006*a*, 2006*b*) as well as enhanced rates of herbicide metabolism occur in Australian resistant ryegrass populations (Matthews *et al*. 1991; Holtum *et al*. 1992; Preston *et al*. 1996; Preston and Powles 1998). Although resistance in some resistant ryegrass populations is due to enhanced ability to metabolise diclofop-methyl, there is not metabolism of sethoxydim (Tardif and Powles 1994). Thus, judicious herbicide choice can give strong indications of prevailing resistance mechanisms, with sethoxydim resistance being a strong indicator of target site resistance to ACCase herbicides (Tardif and Powles 1994).

There were 308 diclofop-methyl resistant populations, with 306 of these populations screened with sethoxydim. Of these, 187 populations (61%) were also found to be resistant to sethoxydim (Table 1). The majority of these populations originated from the medium and higher rainfall zones in the north, H1, M1, M2, and M3 (Table 2). Thus, at least 61% of the ACCase herbicide resistant populations are likely to have target site resistance, i.e. an insensitive ACCase (and possibly some also have a non-target-site based resistance mechanism). Diclofop-methyl and sethoxydim resistance can be endowed by mutations at the amino acid position 1781 (Delye *et al*. 2003; Menchari *et al*. 2006; Zhang and Powles 2006*a*) and positions 2078 and 2088 (Q. Yu, A. Collavo, M. Zheng, M. J. Owen, M. Sattin, S. Powles, unpubl.) in the ACCase gene. As ryegrass is a highly genetically diverse, obligate cross-pollinated species, it is expected that resistant populations comprised individuals carrying different resistance-endowing ACCase gene mutations. Six different ACCase mutations are known to endow ACCase resistance (Delye 2005) and we expect that all possible mutations would be enriched in resistant ryegrass populations.

Of the 306 diclofop-methyl resistant populations, 119 populations (39%) were susceptible to sethoxydim (Table 1). These populations could have ACCase gene mutations that give resistance to diclofop but not to sethoxydim (Delye 2005; Zhang and Powles 2006*a*). Conversely, or additionally, these populations could exhibit non-target-site resistance to diclofop-methyl, due to enhanced P450 enzyme based metabolism. As stated above, enhanced rates of herbicide metabolism in ryegrass can endow resistance to diclofop-methyl but not to sethoxydim (Tardif and Powles 1994).

Resistance to the cyclohexanedione ACCase herbicide clethodim

Clethodim is used for the selective control of ryegrass in dicot crops only (lethal to wheat and barley). Clethodim often controls ryegrass populations resistant to other herbicides. The

Table 2. The percentage of annual ryegrass populations that are resistant (R) or developing resistance (DR) to herbicides, and total resistance (TR) in each agronomic zone (refer to Fig. 1 for agronomic zones)

Zone	Diclofop			Sethoxydim			Clethodim			Sulfometuron			Trifluralin			Atrazine			Glyphosate		
	DR	R	TR	DR	R	TR	DR	R	TR	DR	R	TR	DR	R	TR	DR	R	TR	DR	R	TR
H1	28	62	90	15	69	84	21	0	21	24	69	93	21	0	21	0	0	0	0	0	0
M1	29	71	100	18	68	86	25	4	29	19	81	100	18	0	18	0	0	0	0	0	0
L1	62	15	77	5	35	40	0	0	0	8	92	100	8	0	8	0	0	0	0	0	0
H2	29	46	75	10	57	67	14	0	14	37	48	85	11	0	11	0	0	0	0	0	0
M2	24	70	94	10	68	78	31	3	34	3	97	100	21	0	21	0	0	0	0	0	0
L2	47	38	85	16	40	56	3	0	3	0	100	100	19	0	19	0	0	0	3	0	3
H3	33	22	55	7	27	34	0	0	0	33	19	52	19	0	19	4	0	4	0	0	0
M3	28	58	86	10	65	75	8	0	8	20	77	97	31	0	31	5	0	5	3	0	3
L3	27	38	65	0	41	41	4	0	4	8	92	100	23	0	23	0	0	0	0	0	0
H4	21	6	27	0	22	22	0	0	0	18	15	33	45	3	48	0	0	0	0	0	0
M4	28	34	62	10	40	50	3	0	3	12	88	100	28	0	28	3	0	3	0	0	0
L4	34	17	51	0	53	53	0	0	0	7	93	100	52	0	52	0	0	0	4	0	4
H5	24	24	48	9	45	54	4	0	4	51	31	82	24	0	24	2	0	2	0	0	0
M5	31	41	72	13	48	61	3	0	3	22	69	91	9	0	9	0	0	0	0	0	0
L5	20	0	20	0	0	0	0	0	0	21	79	100	27	0	27	0	0	0	0	0	0

1998 survey found that almost all ryegrass populations remained clethodim susceptible (Llewellyn and Powles 2001). Similarly, in this survey, clethodim controlled 92% of the 451 treated ryegrass populations (Table 1), confirming field experience that this herbicide has retained widespread efficacy. However, 8% of the ryegrass populations contained plants that survived the recommended rate of clethodim and 2 populations were classed as clethodim resistant (Table 1). As resistance to herbicides such as diclofop and chlorsulfuron has not led to cross-resistance to clethodim, resistance has remained at low levels. Selection for clethodim resistance has been relatively low because its use is limited to dicot crops. Populations with clethodim-resistant plants were collected from the higher and medium rainfall zones in the northern agricultural area (Fig. 1; H1, M1, H2, and M2 zones). Clethodim use and consequently resistance is likely to be more frequent in these zones with higher intensity of dicot crops, e.g. lupins.

Resistance to ALS herbicides

Almost 90% of ryegrass populations collected from the WA wheatbelt were found to contain individuals exhibiting resistance to the ALS herbicide sulfometuron. Table 1 reveals that 388 of the 441 ryegrass populations (88%) exhibited resistance to sulfometuron (68% resistant and 20% developing resistance). In general, there was widespread resistance, with over half (51%) of the populations showing greater than 60% survival. While ACCase and ALS herbicide resistance has evolved to the point that resistant annual ryegrass is more common than susceptible ryegrass, ALS herbicide resistance appears to be more abundant (Table 1). In addition to the high initial frequency in ryegrass of mutations endowing ALS herbicide resistance (Preston and Powles 2002*b*), continued use of low-cost ALS herbicides to control susceptible dicot weed species in fields with ALS-resistant ryegrass has further exacerbated resistance to this herbicide group. ALS herbicide resistance in ryegrass was found to be widespread across all agronomic zones. In the H3 and H4 zone, 42% of populations were resistant to sulfometuron (Table 2), whereas in all other zones more than 90% of ryegrass populations were resistant to sulfometuron. Susceptible populations were located only in zones of low cropping intensity (H3, H4).

Multiple resistance

Of the 441 populations that were screened with both the ACCase-inhibitor herbicide diclofop-methyl and the ALS-inhibitor herbicide sulfometuron, 64% of the populations were multiple resistant to both herbicides (Table 3). As described above, insensitive ACCase and ALS enzymes (target sites) are likely to be the resistant mechanisms simultaneously occurring in these multiple-resistant populations, although the presence or extent of non-target-site mechanisms cannot be identified from herbicide screening alone.

There was a significant relationship between presence of resistance to both diclofop-methyl and sulfometuron as indicated in the chi-square test (Table 4). The gamma and tau-b measures of association commonly used for ordinal data indicate that the association is positive, e.g. populations that are resistant to one of the herbicides have a higher probability of being resistant to the other. Only a small number (2%) of diclofop-methyl resistant plants were susceptible to sulfometuron, whereas 24% of sulfometuron resistant plants were susceptible to diclofop-methyl. This suggests that fields with sulfometuron resistance (ALS) are more likely to have ACCase herbicide susceptible plants than *vice versa*.

Table 3. Percentage of multiple-resistant (resistant to both ACCase-(diclofop-methyl) and ALS- (sulfometuron) inhibiting herbicides) populations, single resistance (resistant to either ACCase or ALS herbicides), and susceptible populations in each agronomic zone

Zone	Multiple	Single	Susceptible
H1	86	10	3
M1	100	0	0
L1	75	25	0
H2	70	19	11
M2	97	3	0
L2	83	17	0
H3	41	26	33
M3	83	17	0
L3	68	32	0
H4	15	30	55
M4	63	38	0
L4	54	46	0
H5	42	47	11
M5	69	25	6
L5	14	86	0
Av.	64	28	8

Table 4. Multiple herbicide resistance to both the ACCase herbicide diclofop-methyl and the ALS herbicide sulfometuron

Chi-square = 58.8, 4 d.f., $P < 0.001$, Gamma = 0.20, Kendall's tau-b = 0.12

Sulfometuron	Diclofop			
	R	DR	S	Total
R	137	92	71	300
DR	24	30	34	88
S	4	10	39	53
Total	165	132	144	441

The majority of the multiple-resistant ryegrass populations came from the northern and central-eastern wheatbelt. Areas in the H3 and H4 (Fig. 1) agronomic zones had fewer resistant populations, with H4 having only 15% of populations with multiple resistance. Of the populations in the northern wheatbelt (H1, M1, and L1), 87% had multiple resistance to the ACCase and ALS herbicides (Table 3).

Continued evolution of resistance between 1998 and 2003

The 1998 survey (Llewellyn and Powles 2001) found resistance levels to be high to ACCase and ALS herbicides (diclofop-methyl and chlorsulfuron) in ryegrass across 8 agronomic zones of the WA wheatbelt. Here, we demonstrate that from 1998 to 2003 there has been further increase in the frequency of resistance. The zones surveyed in 1998 were zones H2, M2, L2, M3, L3, H4, M4, and L4 (Fig. 1), and for the purpose of this comparison, only the zones that were sampled in both the 1998 and 2003 surveys are compared. Of the populations tested for resistance to both ACCase and ALS inhibiting

herbicides in both the 1998 and 2003 surveys, the proportion of resistant populations was found to be considerably higher in 2003 than in 1998, with corresponding declines in the proportions of susceptible populations (Table 5). A 2-sample Wilcoxon rank-sum (Mann–Whitney) test confirmed that the distribution of resistance was significantly different between these periods ($P < 0.001$). The proportion of populations with multiple resistance also increased between 1998 and 2003.

The 1998 survey established that 46% of the populations in these zones were classified as diclofop-methyl resistant or developing resistance (Llewellyn and Powles 2001). Over the subsequent 5-year period, this had increased to 68%. Similarly, the proportion of populations with resistance to ALS (sulfonylurea) inhibiting herbicides increased from 64% to 88% over the 5-year period 1998–2003. The spread of resistance through gene mobility (seed and/or pollen) is possible, with many growers perceiving mobility of resistance as a major factor in the occurrence of resistance on their properties (Llewellyn and Allen 2006). However, given the high levels of use of these herbicides, it is assumed that in-field selection is the dominant factor explaining this increase in resistance. Continued selection from ACCase and/or ALS inhibiting herbicide use occurred throughout this 5-year period and, consequently, resistance has continued to evolve to a situation where susceptibility is now rare.

Resistance to triazine herbicides

Ryegrass populations resistant to triazine herbicides are known in WA (Burnet *et al*. 1991) and elsewhere in the Australian wheatbelt (Burnet *et al*. 1994*a*; Broster and Pratley 2006). However, despite the lengthy use of triazine herbicides in WA, only low levels of atrazine resistance were identified in this random survey. Of the 425 populations screened with atrazine, only 5 populations (1%) were classed as developing resistance to atrazine (Table 1). These populations had low-level resistance and came from the medium to higher rainfall zones in the central and southern wheatbelt (H3, M3, M4, and H5C) (Fig. 1). These populations were also resistant to the ACCase and ALS-inhibiting herbicides diclofop-methyl and sulfometuron. These results establish that triazine herbicides remain largely effective across the WA wheatbelt. This may be partly attributed to the use of triazine herbicides on a rotational basis when canola and lupins are grown; meaning that the use of triazines has been relatively low compared with the ACCase and ALS herbicides.

Resistance to dinitroaniline herbicides

Trifluralin is a dinitroaniline herbicide widely used in Australian cropping. The great majority of Australian cropping (no-till) systems use this pre-emergent herbicide for ryegrass control. This reliance on trifluralin for ryegrass control in cropping is now reflected in the evolution of trifluralin resistance. Of the 452 ryegrass populations tested, less than 1% were trifluralin resistant (Table 1). However, importantly, 24% of the populations exhibited trifluralin-resistant individuals and were classed as developing resistance. All populations with trifluralin resistance were also resistant to the ALS-inhibiting herbicides and nearly all populations exhibited resistance to the ACCase-inhibiting herbicides with the exception of some populations in the H4 zone. Resistance to dinitroaniline herbicides has previously been reported in several populations of ryegrass collected from southern Australian dryland cropping systems (McAlister *et al*. 1995). Recent surveying of trifluralin-resistant ryegrass populations in other Australian states (South Australia and Victoria) has detected many populations exhibiting trifluralin resistance (P. Boutsalis and C. Preston, pers. comm.).

Resistance to glyphosate

Despite glyphosate being widely used throughout the wheatbelt, this herbicide has retained efficacy. Of the 432 populations screened with the herbicide glyphosate, only 3 populations (<1%) were classed as developing resistance. These populations were resistant to both the ACCase and ALS-inhibiting herbicides diclofop-methyl and sulfometuron. Although glyphosate-resistant ryegrass has been documented in WA (Neve *et al*. 2004) and elsewhere in Australia (Preston 2005, reviewed in Powles and Preston 2006; Broster and Pratley 2006), in this random survey of crop fields, glyphosate remained effective on almost all of the ryegrass populations.

Comparisons with other studies

The frequency of resistance in ryegrass found in this random survey over the WA wheatbelt is remarkably similar to those reported in a recent paper that summarises the past decade of commercial testing for extent of herbicide resistance in ryegrass in samples submitted from across Australia (Broster and Pratley 2006). This study differs from our random survey in that the seed samples tested were suspected of being herbicide resistant because they were submitted by farmers to a commercial herbicide resistance testing service for evaluation. Testing of seed samples submitted in this way over the past 10 years revealed that, nationally, ACCase and ALS herbicide resistance was confirmed in most ryegrass populations submitted for testing. Clethodim and trifluralin resistance was evident, but at lower levels, and glyphosate resistance was very rare (Broster and Pratley 2006). Thus, there was a good agreement between

Table 5. Change in ALS herbicide (sulfonylurea, SU) and ACCase herbicide (diclofop-methyl, FOP) and clethodim (DIM) resistance status between 1998 and 2003 of populations from the same areas (% of populations tested)

	1998			2003		
	Resistant	Developing	Susceptible	Resistant	Developing	Susceptible
Diclofop	23	23	54	37	31	32
Clethodim	0.5	0	99.5	0.5	7.5	92
Sulfonylurea	38	26	36	68	20	12

the results obtained in this national herbicide resistance testing service and the random survey we conducted over the large WA wheatbelt.

Conclusions

This random survey of 500 fields across 14 million ha of the WA wheatbelt found that most fields were infested with multiple (ACCase + ALS inhibitor)-resistant ryegrass (at least extending across diclofop-methyl, sethoxydim, and sulfometuron). Thus, in less than 25 years, ryegrass in the WA wheatbelt has evolved from being susceptible to being resistant to many herbicides. Although several herbicides (clethodim, triazines, trifluralin, glyphosate) remain effective on ryegrass, evolution of resistance to these important herbicides is underway. Fortunately, despite the widespread resistance to ACCase and ALS inhibiting herbicides, several herbicides (glyphosate, paraquat, clethodim, triazines, trifluralin) continue to be effective on the majority of ryegrass populations. The challenge is to sustain the efficacy of these herbicides through more diversified cropping/farming systems with less herbicide use and integrated management strategies.

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