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Multiple herbicide-resistant *Lolium rigidum* (annual ryegrass) now dominates across the Western Australian grain belt

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Summary

Lolium rigidum (annual or rigid ryegrass) is a widespread annual weed in cropping systems of southern Australia, and herbicide resistance in *L. rigidum* is a common problem in this region. In 2010, a random survey was conducted across the grain belt of Western Australia to determine the frequency of herbicide-resistant *L. rigidum* populations and to compare this with the results of previous surveys in 1998 and 2003. During the survey, 466 cropping fields were visited, with a total of 362 *L. rigidum* populations collected. Screening of these populations with the herbicides commonly used for control of *L. rigidum* revealed that resistance to the ACCase- and ALS-inhibiting herbicides was common, with 96% of populations having plants resistant to the ACCase herbicide diclofop-methyl and 98% having plants resistant to the ALS herbicide sulfometuron. Resistance to another ACCase herbicide, clethodim, is increasing, with 65% of populations now containing resistant plants. Resistance to other herbicide modes of action was significantly lower, with 27% of populations containing plants with resistance to the pre-emergent herbicide trifluralin, and glyphosate, atrazine and paraquat providing good control of most of the populations screened in this survey. Ninety five per cent of *L. rigidum* populations contained plants with resistance to at least two herbicide modes of action. These results demonstrate that resistance levels have increased dramatically for the ACCase- and ALS-inhibiting herbicides since the last survey in 2003 (>95% vs. 70–90%); therefore, the use of a wide range of integrated weed management options are required to sustain these cropping systems in the future.

Keywords: survey, herbicide resistance, diclofop-methyl, glyphosate, trifluralin, sulfometuron.

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Introduction

Global grain crops (including wheat, rice, maize, soya bean and oilseed rape) are crucial to world food supply, and any threats to their productivity endanger food security. Crop-infesting weed species are a major threat to crop productivity. Herbicides can be highly effective and dominate weed control in field crops in all developed nations and increasingly in developing nations. However, a biological repercussion of over-reliance on herbicides is the evolution of herbicide-resistant weed populations. Herbicide-resistant biotypes from 221 weed species have been documented worldwide (Heap, 2013).

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Lolium rigidum Gaudin (annual or rigid ryegrass) is the most widespread and problematic weed of crops in southern Australia. Originally widely planted as a pasture plant (Kloot, 1983), *L. rigidum* is now present across most of the southern Australian cropping region (Gill, 1996) and is a major crop weed which competes with the crop for nutrients and water (Palta & Peltzer, 2001). Control of *L. rigidum* has been achieved by the use of herbicides since the 1970s. Diclofop-methyl and chlorsulfuron were, respectively, the first acetyl coenzyme A carboxylase (ACCase)-inhibiting and acetolactate synthase (ALS)-inhibiting herbicides used in the Western Australian (WA) grain belt (Powles & Howat, 1990). Resistance to ACCase- and ALS-inhibiting herbicides was first reported in the early 1980s (Heap & Knight, 1982; Christopher *et al.*, 1991), with populations exhibiting complex resistance patterns (multiple and cross-resistant) across several herbicide chemistries (Heap & Knight, 1986; Tardif *et al.*, 1993; Burnet *et al.*, 1994b). To date, *L. rigidum* resistance has been reported across 11 herbicide modes of action in 12 countries (Heap, 2013), with surveys in Australia, Spain and the United States revealing the widespread occurrence of herbicide-resistant *Lolium* spp. (Owen *et al.*, 2007; Loureiro *et al.*, 2010; Rauch *et al.*, 2010).

In cropping regions of Australia, there are considerable data on both the area infested with herbicide-resistant *L. rigidum* populations and the herbicides to which resistance exists. In 1998, a random survey of crop fields across the 12 million hectare WA grain belt established widespread *L. rigidum* resistance to the ACCase- and ALS-inhibiting herbicides (Llewellyn & Powles, 2001). Five years later (2003), a second major survey revealed that resistance to the ACCase- and ALS-inhibiting herbicides had increased and resistance to other herbicide modes of action was identified (Owen *et al.*, 2007). Here, we report on a third (2010) large-scale survey of *L. rigidum* across the WA grain belt to update and quantify the geographical extent and spectrum of herbicide resistance.

Materials and methods

Seed collection

Seed material was collected across the Western Australian grain belt from crop fields just prior to grain harvest during October and November 2010 (Fig. 1). Prior to seed collection, farmers were contacted for permission to visit their farms. Farmers from all regions of the grain belt were randomly sourced by attending field days and using local agronomists, grower groups and emails/mail outs within the industry. Farmers provided farm maps or detailed directions to their farms, and, on arrival, crop fields at each farm were chosen at random by sampling the first crop seen after locating the property. They were sampled by two people walking in an inverted ‘V’ pattern across the field. Mature weed seeds from a large number of plants were collected by hand and bulked at the time of collection (see Owen *et al.*, 2007 for detailed sampling methodology). During sampling, weed density was recorded. In total, 466 crop fields were visited, and 362 *L. rigidum* seed samples were collected. In December, seed heads were threshed, and chaff material was separated by aspiration to obtain a clean seed sample. Seed samples were stored in a non-air-conditioned glasshouse from December 2010 to April 2011 to relieve seed dormancy (Steadman *et al.*, 2003).

Fig. 1 Map of south-western Western Australia showing the agronomic zones of the grain belt where *Lolium rigidum* populations were collected for herbicide resistance screening. Annual rainfall isohyets are shown. Rainfall regions are shown by H (high, 450–470 mm), M (medium, 325–450 mm) and L (low, <325 mm). Zones are indicated by 1 (north), 2 (north-central), 3 (central), 4 (south-central) and 5 (south).

Seed Germination

In the following growing season (May–October 2011), seeds from each *L. rigidum* population were placed in 250-mL plastic containers containing 1% agar-solidified water in a growth cabinet set at 25/15°C (light/dark, 12 h daily photoperiod of 30–60 μmol m^{-2} s^{-1}, cool white fluorescent light) for 6 days. When coleoptiles were at a height of approximately 2 cm, 50 seedlings from each population, for each herbicide, were transplanted into plastic seedling trays containing potting mix (50% composted pine bark, 25% peat and 25% river sand). For the pre-emergent herbicide trifluralin, seeds were transplanted when the radicle was just visible, to ensure that all 50 seeds sprayed were viable. For the herbicide atrazine, seedlings were transplanted into seedling trays containing 50% sand and 50% Gingin loam, rather than potting mix, to maximise herbicide activity.

Herbicide resistance screening

Herbicide resistance status was determined by treating 2- to 3-leaf stage seedlings (except those treated with atrazine, which were sprayed when the second leaf was just visible and trifluralin, which was applied pre-emergence) with herbicides using the upper recommended field rates for *L. rigidum* in Australia (Table 1). When seedlings had reached the correct leaf stage, they were treated with a particular herbicide together with the appropriate adjuvant using a custom-built sprayer dual nozzle (TeeJet® XR11001 flat fan, Spraying Systems Co, Wheaton, IL, USA) cabinet sprayer delivering herbicide in 100 L ha^{-1} water at 200 kPa, at a speed of 3.6 km h^{-1}. Herbicide resistance status was tested for the ACCase-inhibiting herbicides diclofop-methyl (Hoegrass, 375 g a.i. L^{-1}, Nufarm, Australia), sethoxydim (Sertin 186 g a.i. L^{-1}, Bayer CropScience, Australia) and clethodim (Select 240 g a.i. L^{-1}, Sumitomo Chemical, Australia); the ALS-inhibiting herbicide sulfometuron-methyl (Oust 750 g a.i. KG^{-1}, DuPont, Australia) was tested and also trifluralin (Triflur X 480 g a.i. L^{-1}, Nufarm, Australia), atrazine (Nutrazine 900 g a.i. L^{-1}, Nufarm, Australia), paraquat (Gramoxone 250 g a.i. L^{-1}, Syngenta, Australia) and glyphosate (Roundup Powermax 540 g a.i. L^{-1}, Nufarm, Australia).

For clethodim, two rates (60 and 120 g a.i. ha^{-1}) were used (Table 1) to reflect the increase in label rate since the previous survey in 2003. As with our previous survey (Owen *et al.*, 2007), diclofop-methyl survivors were cut-back to a height of 1 cm, allowed to regrow and were sprayed with sethoxydim. This was carried out to be consistent with the 2003 survey, as previous work has shown that *L. rigidum* can metabolise diclofop-methyl but not sethoxydim (Tardif & Powles, 1994); therefore, resistance to sethoxydim can indicate target-site resistance to ACCase inhibitors.

Plant mortality was assessed 21 days after treatment, by determining whether the growing point was chlorotic or new growth was visible, as well as comparing with the control populations. For those populations where a small number of individuals survived treatment, these plants were cut-back and resprayed to confirm that the individual plant was resistant. Known susceptible and resistant *L. rigidum* biotypes were used as controls in all experiments, with 100% control of the known susceptible population and high survival (>90%) of the known resistant populations (data not shown). Herbicide treatments were repeated during the growing season, and results averaged for each population (always <5% variation between experiments). In cases where the seed quantity for a particular population was low, some herbicides were omitted and not all herbicide treatments were repeated. Note that resistance screening was conducted under 'ideal' conditions, which are not always experienced in the field; thus, herbicide efficacy in the field may be lower than was observed in this survey.

Data analysis

Resistance has here been defined as a plant's ability to survive herbicide treatment at Australian recommended field rates, which give total control of a well-documented susceptible population (VLR1) (see Owen *et al.*, 2007; Owen & Powles, 2010). Populations have been classified based on the number of individual plants surviving each herbicide treatment. Susceptible

Table 1 Herbicides and rates used for resistance screening of *Lolium rigidum* populations collected in 2010 from the Western Australian grain belt

Herbicide chemical class	Herbicide mode of action	Active ingredient	Field rate (g ha^{-1})
Aryloxyphenoxypropionate	Inhibition of ACCase	Diclofop-methyl	500
Cyclohexanedione	Inhibition of ACCase	Sethoxydim	186
	Inhibition of ACCase	Clethodim	60
	Inhibition of ACCase	Clethodim	120
Sulfonylurea	Inhibition of ALS	Sulfometuron	15
Triazine	Inhibition of photosystem II	Atrazine	900
Dinitroaniline	Inhibition of microtubule assembly	Trifluralin	960
Bipyridyl	Inhibition of photosystem I	Paraquat	250
Glycine	Inhibition of enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)	Glyphosate	540

populations were classified as those having 0% plant survival and resistant populations were classified into two groups: those having 0 < × < 20% plant survival (hereafter denoted as <20%) and those having ≥20%. This classification system, as well as the fact that identical methodology to previous surveys was used, enables comparisons with the previous surveys (Llewellyn & Powles, 2001; Owen *et al.*, 2007) and reflects a management-relevant system of classification, as farmers often visually recognise resistance at a level of around 20% survival, at which point they may stop using the herbicide or consider alternative management options (this visual assessment is dependent on *L. rigidum* densities in the field). Results have been discussed as the number of populations with resistant plants surviving and have been compared with the previous surveys by employing the two categories of resistance described above. These categories of lower and higher resistance define the proportion of plants surviving herbicide application, not the level of resistance of an individual plant; populations in the lower category can still contain highly resistant individuals capable of surviving higher than the recommended rates and going on to produce (resistant) seed.

Results

Cropping spectrum in the WA grain belt

This study surveyed 466 randomly selected cropping fields in which a variety of crop types were grown. Wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and oilseed rape (*Brassica napus* L.) were the most common crops, comprising 65%, 12% and 10% of cropped fields respectively. The minor crops included lupin (*Lupinus* spp.) (6%), oat (*Avena sativa* L.) (4%), field pea (*Pisum sativum* L.) (2%) and chick pea (*Cicer arietinum* L.) (one crop). This result was similar to the 2003 survey, except for a 10-fold increase in oilseed rape. Oilseed rape and oats were more commonly found in the high rainfall zones, while field pea and barley were more common in the southern regions (Fig. 1). Crop fields were visited just prior to harvest, and therefore, weed control practices would have been used earlier in the season; however, some fields may not have been treated due to dry conditions at the start of the 2010 season. At crop maturity, the average weed density of *L. rigidum* was low: only 5% of fields had densities of greater than 10 plants m^{-2}, 17% had densities of 1–10 plants m^{-2} and 42% of crop fields had less than 1 plant m^{-2}. *Lolium rigidum* was difficult to find in 26% of fields and no *L. rigidum* plants were found within the sampling area in 10% of fields.

Resistance to commonly used ACCase-inhibiting herbicides

This survey revealed widespread and high levels of resistance to the commonly used ACCase-inhibiting herbicides (Table 2). The majority (260 of 361) of tested populations exhibited ≥20% plant survival to diclofop-methyl, while a further 85 populations had <20% survival (Table 2). The few susceptible (0% survival) populations (16) were only present in the high rainfall zones and southern cropping regions (Table 3).

All plants that survived treatment with diclofop-methyl were subsequently treated with sethoxydim. Of the 345 *L. rigidum* populations with plants surviving diclofop-methyl, 254 populations had ≥20% plant survival to sethoxydim, while a further 31 had <20% survival (Table 2). Thus, most populations contained individuals which were able to survive both diclofop-methyl and sethoxydim, establishing that resistance extends across the aryloxyphenoxypropionate and cyclohexanedione classes of ACCase-inhibiting herbicides (Table 2).

For the cyclohexanedione-class ACCase-inhibiting herbicide clethodim, two rates were used to screen for resistance. At the 60 g ha^{-1} rate also used in 2003, 113 of 356 tested populations had ≥20% plant survival, and a further 118 populations had <20% survival (Table 2). These populations were found in the medium and high rainfall areas across the WA grain belt (Table 3). At the higher (and currently recommended) rate of 120 g ha^{-1}, 48 populations had ≥20% survival, while 101 had <20% survival (Table 2). The populations with a higher proportion of plants surviving were found mostly in the northern agricultural regions and the higher rainfall regions in the south (Table 3).

Of the 361 populations tested with the different classes of ACCase-inhibiting herbicides in this survey,

Table 2 The percentage of *Lolium rigidum* populations collected in 2010 in each resistance category (fully susceptible, <20% plant survival or ≥20% plant survival) for each herbicide

Herbicide	Susceptible (no survival)	<20% survival	≥20% survival
Diclofop-methyl	4	24	72
Sethoxydim	21	9	70
Clethodim 60 g	35	33	32
Clethodim 120 g	58	28	14
Sulfometuron	2	9	89
Trifluralin	73	26	1
Atrazine	98	2	0
Paraquat	100	0	0
Glyphosate	93	6	1

Table 3 The percentage of *Lolium rigidum* populations in the high-resistance [≥20% surviving plants (H)], low-resistance [<20% surviving plants (L)] or fully susceptible [0% surviving plants (S)] categories by agronomic zone (refer to Fig. 1) for ACCase- and ALS-inhibiting herbicides, glyphosate and trifluralin

Zone	No. Pop's tested	Diclofop-methyl			Clethodim 60 g			Clethodim 120 g			Sulfometuron			Glyphosate			Trifluralin		
		H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
H1	16	63	38	0	40	47	13	13	33	53	81	13	6	0	0	100	0	44	56
M1	28	93	7	0	57	43	0	32	29	39	96	4	0	0	0	100	0	36	64
L1	20	35	50	15	0	30	70	0	0	100	100	0	0	0	0	100	0	55	45
H2	18	83	17	0	53	35	12	39	22	39	89	11	0	0	0	100	0	28	72
M2	27	100	0	0	67	33	0	27	54	19	100	0	0	0	0	100	4	41	56
L2	19	42	53	5	16	21	63	11	11	78	100	0	0	0	0	100	5	21	74
H3	11	82	9	9	27	36	36	0	36	64	55	18	27	0	0	100	0	18	82
M3	43	95	5	0	46	49	5	27	37	37	88	12	0	0	0	100	2	19	79
L3	23	57	39	4	13	22	65	13	4	83	100	0	0	0	0	100	0	13	87
H4	22	68	32	0	36	27	36	9	32	59	59	32	9	0	0	100	0	18	82
M4	36	75	19	6	14	50	36	0	36	64	97	3	0	0	0	100	6	8	86
L4	22	55	41	5	18	18	64	9	5	86	100	0	0	0	0	100	0	41	59
H5	30	70	20	10	23	27	50	3	33	63	80	20	0	7	13	80	0	30	70
M5	37	78	19	3	33	22	44	5	43	51	86	8	5	3	38	59	0	14	86
L5	10	10	60	30	0	11	89	0	11	89	80	20	0	0	44	56	0	20	80

most populations exhibited cross-resistance. Of the 345 populations with plants resistant to diclofop-methyl, 285 populations also had plants resistant to sethoxydim, and 221 had plants resistant to all three ACCase-inhibiting herbicides tested. Clethodim-resistant populations were always resistant to diclofop-methyl, but not always to sethoxydim, and sethoxydim-resistant populations did not always display resistance to clethodim. Only 48 populations contained plants that were resistant to diclofop-methyl alone.

Resistance to a commonly used ALS-inhibiting herbicide

To determine the extent of resistance to the ALS-inhibiting sulfonylurea herbicides, sulfometuron was used, as *L. rigidum* does not metabolise this herbicide (Burnet *et al.*, 1994b), and it therefore provides unequivocal evidence of resistance to this herbicide group. Most populations (323 of 362) exhibited ≥20% plant survival, with the majority having more than 80% survival; a further 31 populations showed a lower proportion (<20%) of plant survival (Fig 2). The only areas to contain populations collected during sampling that were 100% susceptible to the ALS-inhibiting herbicides were in the high rainfall zones (Table 3).

Resistance to other herbicide groups

Only five of 362 populations had a high frequency of plant survival (≥20%) to the pre-emergent herbicide trifluralin, a dinitroaniline-based inhibitor of microtubule production; however, a further 93 populations had a smaller number of plant survivors (<20% survival). These populations were found across the entire WA grain belt (Table 3). The five populations with ≥20% of plants surviving trifluralin were controlled by the alternative pre-emergent herbicides pyroxasulfone and the mixture prosulfocarb + S-metolachlor (data not shown).

No populations had ≥20% plant survival to the photosystem II inhibitor atrazine; however, eight of the 344 populations tested had a smaller number of plants (<20%) surviving (Table 2, Fig 2).

Of the 359 populations evaluated with glyphosate, a non-selective inhibitor of aromatic amino acid synthesis, only three had ≥20% plant survival, while a further 22 populations had plant survival of <20% (Table 2). All populations containing glyphosate-resistant plants came from one region, a southern cropping area extending along the coast between Albany and Esperance (Fig. 1, Table 3). All populations tested were completely susceptible to the alternative burn-down herbicide paraquat (Table 2).

Cross-resistance

The number of populations with resistance to more than one herbicide mode of action was common and was spread across the entire grain belt, with 344 of the 361 tested populations containing plants with resistance to both the ACCase- and ALS-inhibiting herbicides diclofop-methyl and sulfometuron (Table 4). There were 113 populations resistant to ACCase and ALS inhibitors plus one other herbicide (trifluralin or glyphosate), and three populations resistant to all four

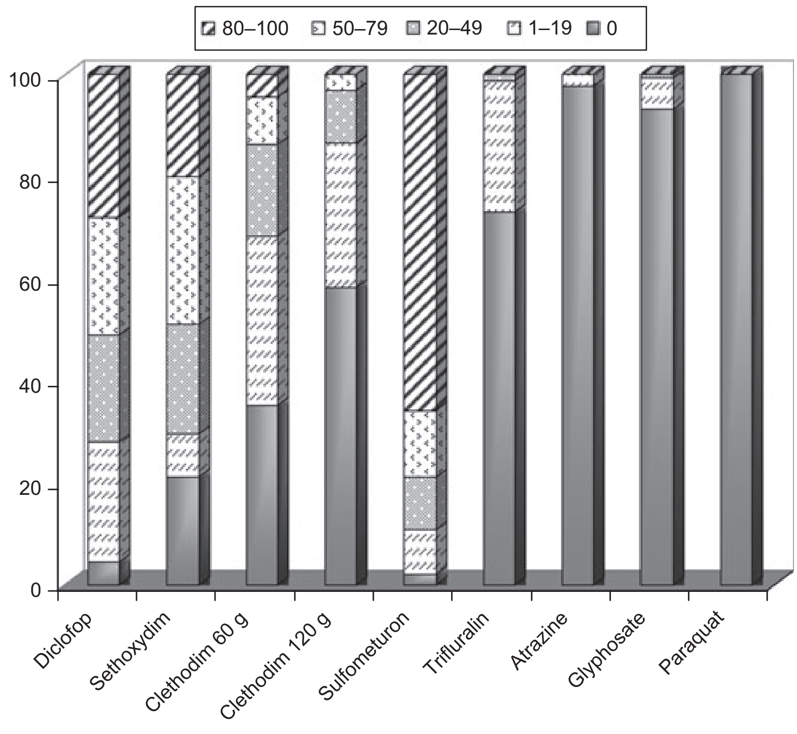

Fig. 2 Severity of resistance to each herbicide tested in 2010. Resistance intervals are 0% (all plants are susceptible), 1–19%, 20–49%, 50–79% and 80–100% of plants surviving each herbicide treatment.

Table 4 The number of *Lolium rigidum* populations with cross-resistance to different herbicide groups and modes of action

Herbicide combination	No. of populations with resistant plants
All herbicides tested	0
ACCase + ALS + trifluralin + glyphosate + atrazine	0
ACCase + ALS + trifluralin + glyphosate	3
ACCase + ALS + trifluralin	93
ACCase + ALS + glyphosate	20
ACCase + ALS	344
ACCase + trifluralin	94
ACCase + atrazine	8
ACCase + glyphosate	22
ALS + trifluralin	96
ALS + atrazine	8
ALS + glyphosate	23

of these herbicide groups (Table 4). There were no populations that contained plants resistant to five, or all six, of the herbicide chemistries tested. Populations with plants resistant to glyphosate, trifluralin or atrazine always had plants that were resistant to the ACCase- and/or ALS-inhibiting herbicides. Of the seeds collected at harvest, there was only one population, from agronomic zone H3 (Fig. 1), that was susceptible to all the herbicides tested in this survey. This zone has a lower cropping intensity and thus probably lower herbicide use than the northern agricultural region.

Resistance evolution 1999–2010

Our previous resistance surveys were conducted in the same region in 1998 and 2003. The 2003 survey revealed an increase by 20 percentage points in the number of populations containing resistant plants for both ACCase- and ALS-inhibiting herbicides since 1998 (Fig. 3). During the 7 years between the 2003 and 2010 surveys, resistance levels to the ALS- and ACCase-inhibiting herbicides have again increased dramatically (by 10 and 28 percentage points, respectively), whereas resistance to the other herbicides such as trifluralin, atrazine and glyphosate has remained relatively steady (Fig. 3). The greatest increase in resistance levels over the 12-year period since the first survey was to the ACCase-inhibiting herbicide clethodim (Fig. 3). In 1998, only one sample was detected as resistant to the label rate of 60 g ha^{-1}; this increased to 8% of populations in 2003 and 65% of populations in 2010 (Fig. 3). Alarmingly, 42% of populations tested in 2010 contained individuals also resistant to the increased label rate of 120 g ha^{-1}.

Discussion

Lolium rigidum is a genetically variable, cross-pollinated crop weed that is widespread in southern Australia because it was previously planted for livestock pasture in many agricultural areas. As cropping replaced pastures, the use of selective post-emergent herbicides to control *L. rigidum* in crops commenced

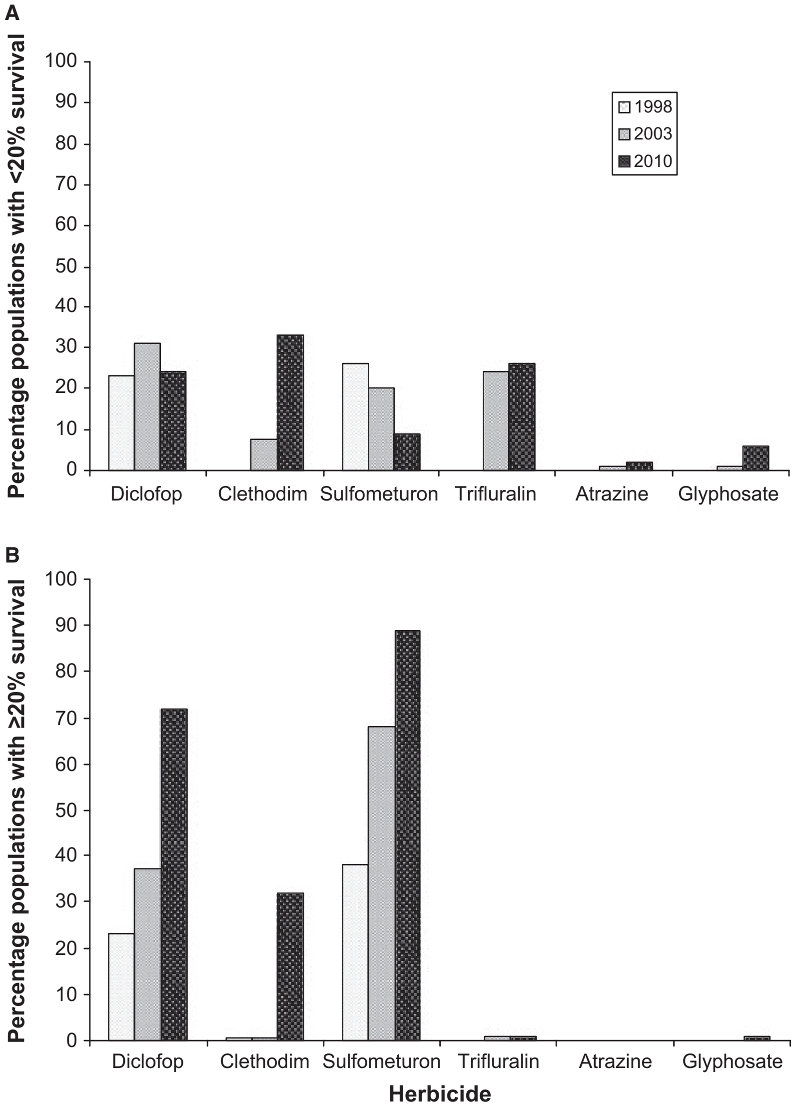

Fig. 3 Changes in herbicide resistance status between 1998, 2003 and 2010 (diclofop-methyl, clethodim (60 g) and sulfometuron) or between 2003 and 2010 (trifluralin, atrazine and glyphosate) for *Lolium rigidum* populations collected from the same regions of the Western Australian grain belt. Resistance values for 1998, 2003 and 2010 include both (A) <20% surviving plants and (B) ≥20% surviving plants, reflecting the categories used by Llewellyn and Powles (2001) and Owen *et al.* (2007).

with the ACCase-inhibiting herbicide diclofop-methyl (from 1978), followed by the ALS-inhibiting herbicide chlorsulfuron (from 1982). The widespread use of these herbicides quickly led to the evolution of herbicide-resistant populations (Heap & Knight, 1982; Christopher *et al.*, 1991). In a survey conducted in 1992/1993, Gill (1995) reported high levels of resistance to both the ACCase- and ALS-inhibiting herbicides for populations collected from Western Australian crop fields that had received more than four applications of each herbicide, establishing that resistance was already common at this time in WA cropping systems.

While most fields sampled in the current survey would have had some herbicide applied in the months prior to the harvest-time seed collection, not all seeds collected were from populations resistant to herbicides (Table 2), illustrating that the timing of seed collection in this survey did not bias the results towards resistance. The fact that herbicides are commonly used in Australia to control weeds on an annual basis (Llewellyn *et al.*, 2012) means that no modern, large-scale survey can be carried out on fields that have not experienced years of herbicide 'pre-selection'. However, although most *L. rigidum* seeds germinate at the beginning of the growing season, a proportion of seeds remain dormant (Owen *et al.*, 2011) and germinate well after the crop has emerged, and therefore, some seeds collected at harvest time may have come from plants that avoided any herbicide pre-selection in that year. It is also important to note that while herbicides resistance was common, only a small number of fields (5%) had greater than 10 plants m^{-2}. These fields may have had no prior weed control at collection and do not necessarily have a high proportion of resistant plants, as shown in our previous surveys where there was no correlation between plant density and the level of herbicide resistance (Llewellyn *et al.*, 2009).

Resistance to ACCase- and ALS-inhibiting herbicides

The current survey confirmed that in the 32 years since the first use of diclofop-methyl, *L. rigidum* populations collected during harvest across the vast WA grain belt have changed from herbicides providing good control to the current situation where almost all fields contain a high number of resistant plants to the in-crop selective ACCase- and ALS-inhibiting herbicides (diclofop-methyl and sulfometuron respectively). This represents an increase of 50 percentage points (i.e. from 46% of populations containing resistant plants to 96%) for diclofop-methyl and 34 points (from 64% to 98%) for sulfometuron, since the first random survey in 1998, when resistance to these herbicides was already quite common (Llewellyn & Powles, 2001). The increase in ACCase and ALS resistance was largely due to the detection of resistant populations in fields from the southern cropping regions. Prior to the 2003 survey, these southern regions were mainly mixed livestock and cropping systems; since that time, these areas have moved towards more continuous cropping (similar to northern cropping areas), possibly increasing the selection pressure for resistance. Thus, *L. rigidum* is now almost uniformly multiple resistant to certain ACCase- and ALS-inhibiting herbicides (Tables 2, 4). A subset of these populations was screened to assess the mechanisms of resistance, and nearly all were found to contain a mix of target-site and non-target-site mechanisms (Yu Q, private communication).

Resistance to a newer ACCase-inhibiting herbicide, clethodim, has become much more widespread in the interval between the 2003 and 2010 surveys (Fig. 3). Although resistance to clethodim was not as frequent as for the other ACCase-inhibiting herbicides, clethodim had the largest increase in the number of populations containing resistant plants (57 percentage points at the 2003 label rate of 60 g ha^{-1}) since the 2003 survey (Fig. 3). In 1998 and 2003, resistant plants were only detected in the northern grain belt in the M2 zone (Fig. 1) (all of these populations had <20% plant survival). However, by 2010, resistance was widespread across the state, with many clethodim-resistant populations detected in the southern growing regions M3, M4, H4 (Fig. 1). Additionally, fields in the M2 zone had shifted to a high number (≥20%) of resistant individuals. Initially, only one mutation in the ACCase gene was known to confer resistance to clethodim (Delye, 2005); it is now known that the particular combination of ACCase mutations present in the plant, the homo- or heterozygosity of a particular mutation and the herbicide rate used can all determine the level of resistance to clethodim (Yu *et al*., 2007). Over time, it is likely that surviving individuals with different resistance mutations/mechanisms cross with each other and enrich the population for resistance. As diclofop-methyl now provides little control for *L. rigidum* in many cropping fields, reliance on clethodim in crops such as lupin and oilseed rape (note that clethodim cannot be used in wheat) can add a greater selection pressure for clethodim resistance. This was observed in the current survey, where clethodim resistance tended to be located in lupin and oilseed rape growing regions (medium and high rainfall zones, Fig. 1) where frequency of use would be higher (Table 3). There was very little clethodim resistance in lower rainfall, marginal areas of the eastern grain belt (zones L1-5, Fig. 1) where these crops are usually not grown and clethodim use would be minimal.

Similar large-scale random surveys on *L. rigidum* resistance conducted in grain-growing regions of other Australian states have also found widespread ACCase- and ALS-inhibiting herbicide resistance (Broster *et al*., 2011; Boutsalis *et al*., 2012). Interestingly, notably lower levels of resistance to these herbicides were found in a recent survey in Tasmania, an island state off the southern coast of mainland Australia that has less intense but more diverse cropping systems with different herbicide use patterns (Broster *et al*., 2012).

The continued increase in resistance in *L. rigidum* to ACCase- and ALS-inhibiting herbicides across Australia (Gill, 1995; Owen *et al*., 2007; Broster *et al*., 2011; current study) is partially because these herbicides are off-patent, low-cost and are used to control a number of other weed species, such as *Avena* spp., *Bromus* spp., *Hordeum* spp. and *Raphanus raphanistrum* L.; therefore, selection pressure on *L. rigidum* has continued. Currently, resistance to ACCase- and ALS-inhibiting herbicides in other grass weed species such as *Bromus* spp. and *Hordeum* spp. is present but still rare (Boutsalis & Preston, 2006; Owen *et al*., 2012a,b). Additionally, while resistance in *L. rigidum* has become common and widespread for the sulfonylurea-class ALS-inhibiting herbicides (Table 2, 3) (see also Gill (1995) and Llewellyn and Powles (2001), who also tested the sulfonylurea herbicide triasulfuron and chlorsulfuron respectively), other ALS-inhibiting herbicide chemistries, such as the imidazolinones, were not tested in this survey and may provide control of some populations that are resistant to sulfometuron.

Resistance to other herbicides

In Australian cropping, there has been widespread reliance on trifluralin as a pre-emergence herbicide for *L. rigidum* control. Trifluralin resistance in *L. rigidum* was first reported 17 years ago (McAlister *et al*., 1995) and is now widespread in the South Australian grain belt (Boutsalis *et al.*, 2012). In the 2003 WA grain belt survey, trifluralin resistance was evident in 24% of fields (Owen *et al.*, 2007) and has not increased much over time (Fig. 2). However, even this relatively low proportion of fields containing plants resistant to trifluralin is of concern. Over-reliance on trifluralin in the face of increasing resistance to other herbicides may accelerate the evolution of resistant populations, as surviving plants cross with each other and possibly stack resistance traits.

The triazine herbicides have a long history of use in Australian agriculture, and resistance to herbicides such as atrazine in *L. rigidum* is known but not widespread (Burnet *et al.*, 1991, 1994a; Broster & Pratley, 2006). Despite their widespread use in oilseed rape and lupin rotations, resistance to triazine herbicides has remained surprisingly low, both in Western Australia (current study) and in New South Wales (Broster *et al.*, 2011). This could be partly due to the fact that triazine herbicides are limited to this phase of the crop rotation.

The number of glyphosate-resistant *L. rigidum* populations reported in the southern WA cropping regions is increasing. In this survey, 25 *L. rigidum* populations contained plants resistant to glyphosate (Tables 2, 3). There have already been a number of documented cases of glyphosate resistance from the southern region (Owen & Powles, 2010). This area often receives summer rainfall, and glyphosate is used to control *L. rigidum* seedlings emerging over the summer fallow months. Therefore, these areas are likely to receive a higher number of applications of glyphosate each year, compared with other areas of the WA grain belt, increasing the selection pressure for resistance. It is possible that by sampling at harvest, the level of glyphosate resistance may be underestimated, as weed control practices used during the season may be controlling plants that are surviving pre-planting glyphosate applications and thus eliminating them from seed collection at harvest. The recent introduction of Roundup Ready oilseed rape in the WA grain belt, and the increasingly common practice of chemical fallowing (using glyphosate), could also increase the selection pressure for resistance to glyphosate. Chemical fallowing is practised in the northern and central WA agricultural areas, so the increased use of this technique may lead to a similar situation to that found in the Liverpool Plains area in New South Wales. Glyphosate resistance is common in this latter area which is dominated by summer cropping and winter fallow and relies on glyphosate for weed control during the fallow period (Storrie & Cook, 2002), thus increasing the selection pressure for resistance. Fortunately, in the current survey, no resistance was detected to the non-selective herbicide paraquat, which can be substituted and rotated with glyphosate in most situations.

Factors influencing the increase in herbicide resistance

In addition to increased reliance on specific herbicides in certain situations and the cross-pollination of individuals with different resistance mutations (discussed above), herbicide resistance spread in *L. rigidum* can also be aided by factors such as (i) long-distance transfer of pollen (gene flow) (Busi *et al.*, 2008); (ii) movement of seeds via livestock (Pleasant & Schlather, 1994) or via machinery during harvest and tillage operations (Blanco-Moreno *et al.*, 2004; Barroso *et al.*, 2006); (iii) seed grain contaminated with weed seeds (Michael *et al.*, 2010). Resistance to ACCase- and ALS-inhibiting herbicides can evolve quickly (3–5 years) in *L. rigidum* (Heap & Knight, 1982; Tardif *et al.*, 1993; Gill, 1995; Powles *et al.*, 1997), and the use of herbicides to control other crop weed species may exert extra selection pressure. Agronomic practices such as crop rotation and tillage also impart selection pressures on weed populations. For example, continuous cropping systems can select for specific weed species populations, whereas diverse systems generally have weed communities with fewer dominant and generally less-troublesome, weed species (Murphy & Lemerle, 2006).

In summary, this set of three random surveys (1998, 2003, 2010) across a 12 million hectare cropping region has revealed that resistance to the ACCase- and ALS-inhibiting herbicides has increased dramatically in the Western Australian grain belt over the past 12 years so that now nearly all fields contain ACCase and ALS resistant individuals. Fortunately, several other mode-of-action herbicides (triazines, trifluralin, glyphosate and paraquat) remain effective, although evolution of resistance to these herbicides is underway. Also encouraging is the fact that while the resistance frequency was high for the ACCase- and ALS-inhibiting herbicides, this and our previous surveys have shown that *L. rigidum* numbers in most fields are relatively low (Llewellyn *et al.*, 2009), meaning that farmers are currently managing them effectively. In Australia, most farmers use crop rotation and a range of herbicide control options and employ weed seed management systems at harvest which aim to reduce the number of seeds entering the soil seed bank. The challenge is to use a wide range of integrated weed management options that help achieve herbicide sustainability and thus productivity of cropping systems.

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