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Herbicide Resistance in Rigid Ryegrass (*Lolium rigidum)* Has Not Led to Higher Weed Densities in Western Australian Cropping Fields

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The aim of this study was to test whether herbicide resistance in rigid ryegrass has led to increased densities of this weed in Western Australian (WA) cropping fields. A total of 503 wheat fields with previously unknown management history and weed status were visited prior to harvest across 15 agronomic areas of the central WA cropping belt in 1998 and 2003. Rigid ryegrass density was visually assessed and, where possible, seed was collected from the population. Ryegrass was found in 91% of the wheat crops sampled. Ryegrass populations were tested in the following year for resistance to chlorsulfuron, sulfometuron, diclofop, and clethodim. With the use of nonparametric and regression statistical methods, resistance status, including multiple-resistance status, was not found to be associated with higher weed density. The results show that growers are generally maintaining low densities in fields with herbicide-resistant rigid ryegrass. The most common rigid ryegrass density at harvest time was less than 1 plant m^{-2} in both resistant and susceptible populations. Field and model-based studies of weed and herbicide resistance management that allow populations to continue at very high densities are unlikely to reflect common grower practice.

Nomenclature: Chlorsulfuron; clethodim; diclofop; rigid ryegrass, *Lolium rigidum* Gaudin LOLRI; wheat, *Triticum aestivum* L.

Key words: Herbicide resistance, management, density, economics, ryegrass.

A common repercussion of high herbicide reliance in cropping systems is the evolution of herbicide-resistant weed populations and the loss of effective use of those herbicides over time (Heap 2008). When faced with the loss of a previously preferred herbicide, growers can strive to avoid increased weed population densities through increasing their use of possibly less cost-effective alternative herbicide and nonherbicide options. Another possible consequence of herbicide resistance is higher weed densities, and therefore reduced crop yields.

Herbicide-resistant weed populations are common throughout the 10-million-ha WA cropping zone (Owen et al. 2007, Walsh et al. 2001). The most common resistant weed is rigid ryegrass, which has multiple resistance to acetyl-Coenzyme A carboxylase (ACCase)- and acetolactate synthase (ALS) –inhibiting herbicides, as well as other herbicide groups (Owen et al. 2007). Rigid ryegrass is the most economically important weed of Australian cropping, as it is common throughout the southern Australian cropping zone (Alemseged et al. 2001).

Economic theory suggests that increased weed control costs (e.g., due to weed resistance to a preferred herbicide) will reduce the economically optimal level of weed control, thereby leading to an increased optimal maximum weed density (Auld et al. 1987; Jones and Medd 2000; Monjardino et al. 2003; Pannell et al. 2004). If being forced to switch to alternative weed control practices leads to only slightly higher costs for the same level of weed control, then the economically optimal maximum weed density is not likely to be substantially increased.

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An important factor leading to low economic maximum weed densities is the dynamic, longer-term nature of the soil seedbank of annual weeds (Cousens 1987). In broadacre Australian cereal grain cropping, Jones and Medd (2000) found the threshold level for control reduced from 39 wild oat (*Avena fatua* L.) seeds m^{-2} to 6 when longer-term weed seed effects on subsequent crops were considered, and threshold levels can approach an effective level of zero (also see Bauer and Mortensen 1992). A simplistic assumption in modeling of weed and herbicide resistance management would be for weed densities to become very high after resistance first develops and continue at high densities through subsequent crop years.

Materials and Methods

Field Survey. Data in this study are drawn from two field surveys described in Owen et al. (2007) and Llewellyn and Powles (2001). Because herbicide-resistant rigid ryegrass was known to be common in the WA cropping belt, the site-selection methodology used in these studies did not involve deliberately selecting fields with a known higher probability of resistance (Beckie et al. 2001; Davis et al. 2008). Instead, a systematic methodology primarily aimed at determining frequency of resistance occurrence in the region was used. A total of 764 cropping fields were visited over two cropping seasons. Two hundred sixty four in-crop fields were visited across eight agronomic areas of the central WA wheat belt in 1998 (regions 4–6 and 8–12 in Figure 1). A larger survey of the WA wheat belt was conducted in 2003 (Owen et al. 2007), in which a further 500 cropping fields from 15 agronomic regions were visited (Figure 1). The surveys were conducted at the end of the growing season before harvest (October to December) after all in-crop ryegrass control practices had been carried out by growers. Growing conditions in 2003 were excellent across most of the major cropping WA regions, resulting in well-above-average yields. In 1998 crop yields were also above average [Australian Bureau of Agricultural and Resource Economics (ABARE) 2006].

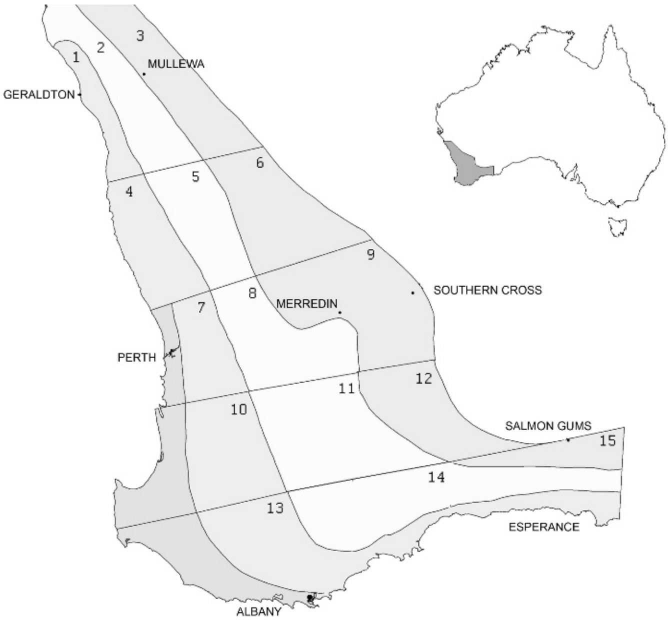

Figure 1. Location of rigid ryegrass seed sampling regions.

The large sample region includes a range of historical cropping intensities and selective herbicide use. Within each of the agronomic areas (Figure 1), at least 30 fields were systematically selected by traveling a predetermined distance (5 km) along minor and major roads and then surveying the nearest in-crop field. A handheld global positioning system unit was used to record latitude and longitude for each site. An area of approximately 5,000 m^2 was surveyed in each field by two people, each following an inverted V-shaped path, beginning no less than 20 m from the edge of the field. Mature rigid ryegrass spikes were collected from plants along the sampling path. If more than 10 seed-producing rigid ryegrass plants were found within the sampling area, the seed sample was retained. If fewer than 10 seed-producing plants were found no sample was retained, as it was considered that too few seedlings for testing may result and/or the seed sample may not satisfactorily represent the plant population in the sample area. Rigid ryegrass density in the sampling area was visually assessed by each sampler along their sampling path. At the completion of sampling an average estimated density for the sampled area was agreed upon with the use of the following categories: Low (< 1 plant m^{-2}), Medium (1–10 plants m^{-2}), High (> 10 plants m^{-2}) and Very High (> 10 plants m^{-2}; ryegrass dominating crop).

The vast majority of surveyed fields were in crop to wheat (523). Due to the low number of observations from crops other than wheat, only data from wheat fields were used in the statistical analysis of association. An adequate sample of mature rigid ryegrass seed was collected from 453 of wheat fields visited. Fields in which no rigid ryegrass population could be sampled for herbicide resistance testing were not included in the density versus resistance status analyses.

Resistance Testing. The resistance testing procedures are described in detail in Owen et al. (2007) and Llewellyn and Powles (2001). In summary, to test for resistance to the ACCase-inhibiting herbicides, populations were treated with the aryloxyphenoxypropanoate herbicide diclofop (375 g ha^{-1} in 1999 and 563 g ha^{-1} in 2004) and the cyclohexanedione herbicide clethodim (48 g ha^{-1} in 1999 and 60 g ha^{-1} in 2004). To test for resistance to the ALS-inhibiting sulfonylurea herbicides, populations in 1999 were treated with chlorsulfuron (30 g ha^{-1}) and a later application of sulfometuron (30 g ha^{-1}) to confirm an ALS target-site–based resistance (Burnet et al. 1994). In 2004, populations were treated with sulfometuron (15 g ha^{-1}). A small number of populations did not achieve satisfactory germination and/or establishment, resulting in less than the number of population samples being tested and included in the analyses.

Populations were classified as Resistant if greater than 20% of rigid ryegrass plants survived the herbicide treatment. Populations in which 1–20% of plants survived were classified as Developing Resistance. Where all plants were killed by the herbicide treatment, the population was classified as Susceptible. An overall resistance score based on the sum of diclofop, sulfonylurea, and clethodim resistance status was also calculated. Based on resistance tests, a score of 2 was awarded for each Resistant classification, 1 for each Developing Resistance classification, and 0 for each Susceptible classification; i.e., populations with a score of 6 are resistant to all three herbicides.

Statistical Analysis. Chi-square contingency table (3 by 4) tests were used to determine if diclofop, clethodim, and sulfonylurea resistance scores (1–3) were associated with weed density classifications. A 6 by 4 chi-square contingency table test was used to determine if multiple resistance score (1–6) was associated with field density classification. Proportional reduction in error measures of association for ordinal measures of resistance and density (Kendall's tau-b) are included to show the direction of association (-1 to 1) for tests with chi-square $P < 0.2$ (see Meddis 1984 for descriptions of tests used). Noninterval variables were analyzed with the use of nonparametric tests. A 2 by 3 chi-square contingency table test was used to identify associations between resistance scores and latitude [i.e., northerly (regions 1–6) and southerly (regions 10–15) regions]. Wilcoxon rank-sum tests were used to determine the significance of changes in diclofop and sulfonylurea resistance scores between years, and the change in overall resistance scores between years in both the northerly and southerly regions.

A series of maximum-likelihood ordered logit regressions (see Long and Freese 2001) were also used on the combined data from both surveys to determine whether the levels of rigid ryegrass resistance amongst different herbicide groups were significant explanatory variables for rigid ryegrass density (as an ordered categorical variable) when other potentially influential variables such as latitude and year of sampling are also included. Four models were estimated, one for each of the three herbicides (diclofop, clethodim, and sulfonylurea), and one for an overall herbicide resistance scale based on the sum of resistance scores (described above). Other independent variables included in the models included latitude ($1 = > -29°50'S$, $2 = -31°20'S$ to $-29°50'S$; $3 = -32°20'S$ to $-31°20'S$; $4 = < -32°20'S$), rainfall ($1 =< 325$ mm, $2 = 325$–450 mm, and $3 = > 450$ mm), and sample year ($0 = 1998$, $1 = 2003$). The dependent variable was based on the visually assessed average weed densities using the classifications of 1 (Low, < 1 plant m^{-2}), 2 (Medium, 1–10 plants m^{-2}), 3 (High, > 10 plants m^{-2}), and 4 (Very High).

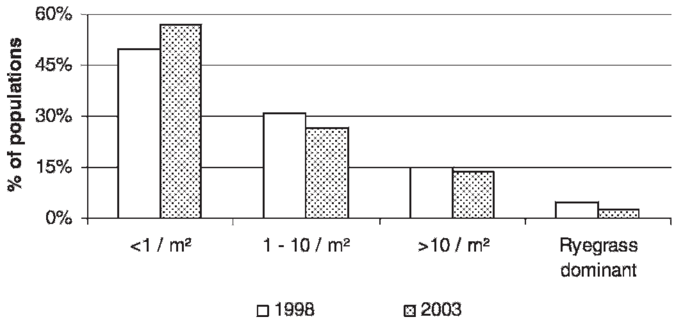

Figure 2. Distribution of ryegrass population densities based on surveys of 523 wheat fields in 1998 and 2003.

This study tests the hypothesis that herbicide resistance in rigid ryegrass is not associated with higher densities of this weed in WA cropping fields. In doing so, we explore whether the major costs conferred by herbicides failing due to resistance are associated with higher densities affecting crop production, or whether farmers have maintained resistant weed populations at relatively low densities. Implications for the biological and bioeconomic modeling of weed and herbicide resistance management are discussed, along with recommendations for research and extension.

Results and Discussion

Extent and Density of Rigid Ryegrass. Rigid ryegrass was found in 91% of the 523 wheat crops sampled. The most common estimated ryegrass density was < 1 plant m^{-2}, and 19% of fields had high (> 10 plants m^{-2}) or very high densities where rigid ryegrass was visually suppressing the crop (Figure 2). The distribution of population densities was strongly skewed toward the low end of the spectrum. The density of rigid ryegrass populations tended to be higher in the southern regions. A chi-square test revealed a significant difference ($P < 0.009$) between the density categories of the northern (regions 1–6) and southern (regions 10–15) regions with lower densities less common in southern regions.

Extent of Resistance to ACCase- and ALS-Inhibiting Herbicides. Of the 442 rigid ryegrass populations tested with the ACCase-inhibiting herbicide diclofop (FOP), 34% were Resistant and 32% were in the Developing Resistance category. The proportion of rigid ryegrass populations with susceptibility, developing resistance, and resistance to sulfonylurea, diclofop, and clethodim is shown in Table 1. When the regions that were sampled in both years were compared, the proportion of FOP-resistant populations was higher in 2003 with a two-sample Wilcoxon rank-sum (Mann-Whitney) test confirming significantly different distributions between years ($P < 0.001$). No clethodim (DIM) -resistant populations were recorded in 1998; however, by 2003, 6% of populations were classified as developing DIM resistance.

Of the 432 populations tested for resistance to the ALS-inhibiting sulfonylurea (SU) herbicide, 9% were susceptible, 19% were developing resistance, and 72% were found to be resistant (Table 1). In the 5 yr between the 1998 and the 2003 survey there was an increase in the proportion of SU-resistant populations and corresponding declines in the proportions of susceptible plants and those showing signs of developing resistance. For regions that were sampled in both years, a two-sample Wilcoxon rank-sum (Mann-Whitney) test confirmed that the distribution of SU resistance status was significantly different between these periods ($P < 0.001$).

Table 1. Proportion (%) of rigid ryegrass populations showing susceptibility, developing resistance, and resistance to sulfonylurea (SU), diclofop (FOP), and clethodim (DIM) herbicides.

	% of populations		
Resistance status	SU	FOP	DIM
Susceptible	9	34	93
Developing resistance	19	32	6
Resistant	72	34	< 1

Overall, 51% of rigid ryegrass populations used in the analyses were resistant to either sulfonylurea or diclofop or both of these herbicides, with a further 26% resistant to one of these herbicides and developing resistance to a second, including clethodim. Multiple resistance (i.e. resistance to both ACCase and ALS herbicides) was observed in 40% of populations. Five percent of the populations tested were resistant to both sulfonylurea and diclofop and developing clethodim resistance.

Resistance Status and Density. Measures of central tendency of field density classification for tested populations showing susceptibility, developing resistance, and resistance to sulfonylurea, diclofop, and clethodim are shown in Table 2. Mean rigid ryegrass density scores ranged from 1.6 to 2.1, and were thus generally in the Medium (1–10 plants m^{-2}) category (Table 2). The median density score was also 2 (1–10 plants m^{-2}) for all levels of resistance to all herbicides apart from populations showing developing resistance to diclofop, which had a lower median of 1 (< 10 plants m^{-2}). The mode density score varied between 1 and 2 among all levels of resistance, apart from diclofop, where the mode was 1 (< 10 plants m^{-2}) for all levels of resistance. Resistance status as measured by combined resistance score was found to be independent of field density [chi-square test, $P = 0.809$, 18 degrees of freedom (d.f.), Table 3]. No association between sulfonylurea, clethodim, and diclofop resistance and higher in-crop densities was found (sulfonylurea: chi-square test, $P = 0.445$, 6 d.f.; clethodim: chi-square test, $P = 0.113$, 6 d.f., Kendall's tau-b = 0.07, diclofop: chi-square test, $P = 0.06$, 6 d.f., Kendall's tau-b = $-$ 0.01, Table 4).

Because of the possible interactions with latitude and year of sampling, multivariate analysis was also applied and is briefly summarized here. Ordered logit regressions confirmed no significant association between the resistance score or diclofop and sulfonylurea resistance status variables and density classification (results not presented). The overall significance of the model describing the effect of clethodim resistance was too low to reach any conclusion. Latitude was significant ($P < 0.05$) in all models, with more southerly latitudes being associated with greater ryegrass densities. Neither average annual rainfall nor year of sampling were associated with rigid ryegrass density in any of the regression models.

The results of this study suggest that the density of rigid ryegrass in WA cropping fields with a herbicide-resistant population is generally not higher than in fields with susceptible populations. Clearly, notwithstanding existing levels of multiple herbicide resistance, growers are controlling ryegrass numbers. Together with alternative herbicides and

Table 2. Density[a] of rigid ryegrass populations susceptible, developing resistance, and resistant to sulfonylurea (SU), diclofop (FOP), and clethodim (DIM) herbicides (based on density score).

	SU[b]			FOP			DIM[c]		
Category	Mean	Median	Mode	Mean	Median	Mode	Mean	Median	Mode
Susceptible	2.0	2	2	1.9	2	1	1.8	2	1
Developing Resistance	1.8	2	1	1.6	1	1	2.1	2	1,2[c]
Resistant	1.8	2	1	1.9	2	1	2.0	2	2

[a] Density score = 1 (Low): < 1 plant/m^2; 2 (Medium): 1–10 plants/m^2; 3 (High): > 10 plants/m^2; 4 (Very high).
[b] Chlorsulfuron resistance status visually assessed in 1999.
[c] Bimodal; only 26 populations were developing resistance to DIMs and 1 population was resistant to DIMs.

crop sequence changes, the suite of ryegrass control practices that are commonly adopted by WA grain growers after herbicide resistance (see Llewellyn et al. 2004) appear to be successful in controlling common forms of herbicide-resistant ryegrass.

It is important to note that the analyses conducted in this study were limited to rigid ryegrass resistant to ALS- and ACCase-inhibiting herbicides and the results should be considered to be case-specific. Resistance to other key herbicides and resistance in other weed species (see Owen et al. 2007 and Walsh et al. 2001), although currently less common, are expected to require greater weed control expenditure (e.g., Llewellyn et al. 2002) and may lead to increased densities. It should also be noted that association between clethodim resistance and density could not be conclusively tested in this study because of the low number of resistant populations. The simple and visual nature of the in-field density assessments may have affected the ability to statistically identify minor associations between density and resistance. However, the primary aim of this paper was to identify substantial associations of practical significance when considering the management response of growers to herbicide resistance. None were found.

The results of this study support the argument that modeling and field-based studies that include only very limited alternative weed management practices as options for herbicide-resistant population management are likely to overstate the effect of herbicide resistance development on managed weed densities and are unlikely to reflect the common grower approach to managing rigid ryegrass and herbicide resistance. At least for common forms of herbicide resistance in rigid ryegrass, the primary cost of herbicide resistance does not appear to be incurred through ongoing higher weed densities in crops. Herbicide-resistant rigid ryegrass populations are typically being maintained at the same low densities as susceptible populations.

Table 3. Density[a] of rigid ryegrass according to resistance score.[b]

Resistance score[c]	% of sample	Mean	Median	Mode
0	7.2	2.0	2.0	2.0
1	9.3	1.8	2.0	1.0
2	27.6	1.8	2.0	1.0
3	26.4	1.7	1.0	1.0
4	24.1	1.9	2.0	1.0
5	5.3	2.0	2.0	1.0

[a] Chi-square statistic = 17.9, 18 d.f., P = 0.81.
[b] Density score = 1 (Low): < 1 plant/m^2; 2 (Medium): 1–10 plants/m^2; 3 (High): > 10 plants/m^2; 4 (Very high).
[c] Score based on the sum of diclofop, chlorsulfuron, and clethodim resistance status, where 2 is awarded for each Resistant classification (> 20% survivors), 1 for each Developing Resistance classification (1–20% survivors), and 0 for each Susceptible classification. Only one population had a score of 6, i.e., resistant to all three herbicides.

Table 4. Density distribution of susceptible (S), developing resistance (DR), and resistant (R) rigid ryegrass populations (percent of populations tested).

		Density			
		< 1 m^{-2}	1–10 m^{-2}	> 10 m^{-2}	Ryegrass dominant
SU[a]	S (*n* = 39)	28	49	15	8
	DR (*n* = 83)	46	33	18	4
	R (*n* = 310)	45	34	17	5
FOP[b]	S (*n* = 153)	38	38	19	5
	DR (*n* = 140)	56	29	11	3
	R (*n* = 149)	40	34	20	5
DIM[c]	S (*n* = 415)	45	34	17	4
	DR (*n* = 26)	35	35	15	15
	R (*n* = 1)	0	100	0	0

[a] SU = sulfonylurea. Chi-square statistic = 5.8, 6 d.f., P = 0.45.
[b] FOP = diclofop. Chi-square statistic = 12.2, 6 d.f., P = 0.06, Kendall's tau-b = − 0.01.
[c] DIM = clethodim. Chi-square statistic = 9.8, 6 d.f., P = 0.11, Kendall's tau-b = 0.07.

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