

Cropping practices influence incidence of herbicide resistance in annual ryegrass (*Lolium rigidum*) in Australia

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Abstract. Herbicide resistance is a common occurrence in southern Australia. The evolution of herbicide resistance is influenced by the selection pressure placed on the weed species controlled by that herbicide. Results from resistance screening of ~4500 annual ryegrass (*Lolium rigidum* Gaud.) samples were entered in a GIS database, together with several agricultural parameters used in the Australian Bureau of Statistics Agricultural Surveys. This allowed a study of the associations between mode of action of resistance, geographic distribution of resistance across southern Australia, and farming practices employed in particular regions.

Cultivation was negatively associated with resistances in acetyl-CoA carboxylase (ACCase)-inhibiting cyclohexanedione and acetolactate synthase (ALS)-inhibiting herbicides. Higher proportions of wheat sown were associated with higher incidences of resistance. ACCase-inhibiting aryloxyphenoxypropionate and cyclohexanedione and ALS-inhibiting resistances were higher in those shires where soils were predominantly acidic. This study demonstrates the association between farm practice and the evolution of herbicide resistance. The analysis provides reinforcement to the principle of rotating chemical modes of action with non-chemical weed control measures to minimise the risk of herbicide resistance evolution in any farming system.

Additional keywords: crop residue, soil pH, tillage.

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Introduction

Herbicide resistance is a common occurrence in weeds of winter crops in southern Australia. In particular, annual ryegrass (*Lolium rigidum* Gaud.), one of the most prevalent weed species across the southern Australian wheat-cropping zone, has evolved resistance to most herbicide modes of action used for its control. These modes of action, as classified by Australian Pesticides and Veterinary Medicines Authority (APVMA), Herbicide Resistance Action Committee (HRAC) and Weed Science Society of America (WSSA), include: APVMA Group A (HRAC Group A, WSSA Group 1), acetyl-CoA carboxylase (ACCase) inhibitors—‘fops’ (aryloxyphenoxypropionates), ‘dims’ (cyclohexanediones) and ‘dens’ (phenylpyrazoles) (Heap and Knight 1982; Heap and Knight 1986; Boutsalis *et al.* 2012); APVMA Group B (HRAC Group B, WSSA Group 2), acetolactate synthase (ALS) inhibitors—‘SUs’ (sulfonylureas) and ‘imis’ (imidazolinones) (Matthews *et al.* 1990; Powles and Howat 1990; Christopher *et al.* 1991); APVMA Group C (HRAC Group C1, WSSA Group 5) inhibitors of photosynthesis at PSII—triazines (Burnet *et al.* 1991; Pratley *et al.* 1993b); APVMA Group D (HRAC Group K1, WSSA Group 3), inhibitors of tubulin

formation—dinitroanilines (Pratley *et al.* 1993b; McAlister *et al.* 1995); and APVMA Group M (HRAC Group G, WSSA Group 9), inhibitors of EPSP synthase—glycines (Pratley *et al.* 1996, 1999; Powles *et al.* 1998). Additionally, as a result of diversity in both its genetics and the management practices used across the southern cropping region, several different patterns of cross-resistance and multiple resistances occur in annual ryegrass (Broster and Pratley 2006).

The efficacy of a particular herbicide to control on-farm populations of annual ryegrass can be determined by a herbicide-resistance test. Long-term data collected through the herbicide-resistance testing service at Charles Sturt University (CSU), Wagga Wagga, have shown extensive variation in the incidence of resistance in annual ryegrass among locations across south-eastern Australia (Broster and Pratley 2006). Similarly, random surveys conducted since 2003 in several states of Australia have shown differences in the incidence of resistance, both between states and between regions within states (Broster *et al.* 2011, 2012, 2013; Boutsalis *et al.* 2012; Owen *et al.* 2014). Compared with the initial surveys in many of the same regions (Pratley *et al.* 1993a; Henskens *et al.* 1996; Nietschke *et al.* 1996; Llewellyn and Powles 2001), the most

recent surveys have recorded changes in the incidence and patterns of herbicide resistance. Although samples for the herbicide-resistance testing service at CSU are obtained directly from farms or farm advisers when resistance is suspected, a comparison with a previous Western Australian random survey (Owen *et al.* 2007) showed good agreement in the frequency of resistance between the two data sources.

The evolution of herbicide resistance is influenced by the selection pressure placed on the weed species controlled by that herbicide. The nature of each combination of herbicide, weed population and resistance status determines the practices that govern the use of different herbicides, thereby influencing the selection pressure exerted by each herbicide (Christoffers 1999). Soil type, management practices and the type of crop grown all influence herbicide use. The persistence of some chemicals can be extended if applied to soils that are acidic (e.g. imidazolinones) or alkaline (e.g. sulfonylureas), thereby limiting crop-rotation options (Black *et al.* 1999; Kennedy 2003). Reduced tillage can result in increased soil acidity, enhancing the persistence of some herbicides or limiting crop rotations through the pH level itself (Blevins *et al.* 1983; Locke and Bryson 1997).

Likewise, the requirement of some herbicides for incorporation after application limits their suitability in reduced-cultivation systems (Parsons 1995). Increased levels of crop residue on the soil surface can also reduce the activity of some herbicides because they bind to the plant residue, physically separating them from the soil where they would be activated, reducing their capacity for use in this situation (Locke and Bryson 1997). Additionally, depending upon the herbicide, persistence can either be increased or decreased in reduced-tillage systems, thereby influencing herbicide carryover effects on subsequent crop rotations (Locke and Bryson 1997).

Cultivation can be used for attaining some weed control and reducing the reliance on herbicides (Llewellyn *et al.* 2004); therefore, the adoption of no-till farming was perceived by farmers in South and Western Australia to lead to increases in herbicide dependence and resistance (D’Emden and Llewellyn 2006).

Regional differences occur across the southern Australian cropping region with respect to crop species grown, rotations adopted and management practices used (ABS 2018). Such differences are due to climatic constraints, soil types, weed problems and availability of suitable cultivars, and are often influenced by regional practices. It is expected, therefore, that management practices would influence the range of herbicides used and thereby indirectly influence the likelihood of herbicide resistance. This paper evaluates the association between the incidence of herbicide resistances across southern Australia and the farming practices adopted on a regional basis.

Materials and methods

Charles Sturt University receives annual ryegrass samples from across the southern cereal-cropping region from November to March each year for resistance testing. The methodology used for testing both post-emergent and pre-emergent herbicides has been described in detail in Broster and Pratley (2006).

Briefly, samples received are threshed, cleaned and stored at room temperature until tested. Just before testing, samples are placed in an incubator for 1 week at 2–5°C to break any dormancy and ensure evenness of germination. Seeds for post-emergent testing are planted into a 50 : 50 peat moss–sand mix, and those for pre-emergence testing are planted into soil. Herbicides are applied at the recommended growth stages by using an automated cabinet sprayer applying volumes equivalent to 83 L ha^{−1} from a flat-fan nozzle at 300 kPa pressure. Rates of 0.5, 1.0 and 2.0 times the recommended label rate are applied to three replicates, and each batch contains known resistant and susceptible biotypes as standards. The results for all sample tests are entered into a geographical information system (GIS) to allow the identification of regional trends.

Agricultural survey data based on each shire were obtained from the Australian Bureau of Statistics (ABS 2018). The data comprised winter crops grown (2001–07 and 2010), amount of cultivation (2001–02 and 2010), and stubble management (2001–02 and 2010). The predominant soil pH for the shire was calculated by using data from the CSIRO Soil Atlas to determine the pH rating (acidic, neutral or alkaline) that held the largest proportion of the shire area (Northcote 1979; McKenzie and Hook 1992). Total shire area and estimated arable area (cropping area plus improved pastures) were also provided.

An analysis was undertaken to evaluate whether the incidence of herbicide resistance could be associated with cropping practices and soil pH. Shires were based on 2015 boundaries and were included only if they fulfilled the conditions of: (i) annual mean area of winter crop >3000 ha; and (ii) minimum of four samples tested for resistance between 2001 and 2015.

The data were processed in two stages to determine annual means for all categories. First, means were determined for the 2001–07 (crops grown) or 2001–02 (cultivation and stubble management) data, and second, the overall mean was determined by the average of this and the 2010 data. The classes of data available for analysis are shown in Table 1. In total, 4539 samples were received from the 173 shires. After removing shires that did not fulfil the criteria, 122 shires remained to provide samples. Only one shire from Tasmania fulfilled the above criteria, and this was also removed from the dataset because, in later analyses, state was considered as a factor. Thus, the final dataset contained 121 shires.

Because the exact characteristics of the farms from which the samples came were unknown, it was assumed that farms matched the predominant characteristics of the corresponding

Table 1. Classes of data available for analysis
Herbicide Groups are APVMA mode-of-action designations

Herbicide Group:	A fops, A dims, B, C, D, M
Crop types:	Wheat, barley, canola,
Cultivation:	None, 1 or 2, >2
Crop residue:	Left intact, mulched, incorporated, removed, total burnt, hot burn, cool burn
Miscellaneous:	Shire pH, samples, arable area, cropping intensity

Table 2. Description of variates used and categorisation for data analyses

Characteristic	Variable	Description
Soil pH	pH	Coded 1 if acidic
State of shire	NSW	Coded 1 if shire is in NSW
	Vic.	Coded 1 if shire is in Vic.
	SA	Coded 1 if shire is in SA
Winter crop	Wheat	Coded 1 if predominant crop is wheat
Amount of cultivation	Cultivation	Coded 1 if predominant number of cultivation is at least one
Crop residue management	Intact	Coded 1 if predominant crop residue management method is 'left intact'
	Incorporated	Coded 1 if predominant crop residue management method is 'incorporated'

shires. Hence, instead of using the numerical values of the variables, these variables were categorised and indicator variables were introduced for model fitting (Table 2). Some classes of the variables that rarely appeared in the dataset were combined to avoid potential biases.

Statistical analyses

The association between farming practices and incidence of herbicide resistance was analysed by using auto-binomial models (Besag 1974, 1975; Johansson 2001), which include the usual logistic regression model as a special case. The usage of auto-binomial models allowed the study of spatial effects along all other variables. The model is briefly described below. The methodology and reasoning behind the statistical procedures chosen are further explained in Ip *et al.* (2018).

In each shire i , it was considered that the number of resistant samples k_i followed a conditional binomial distribution with 'number of trials' n_i , the number of samples tested, and the 'probability of success' $p(x_i, k_j)$, where x_i represented the set of covariates (Table 2) of shire i , and k_j represented the number of resistant samples in the 'neighbourhood'. Beside the characteristics of the shire, the number of herbicide-resistant incidences in shires nearby was also used to model the probability of resistant incidence for that shire. For this, the latitude and longitude at the approximate central point of each shire were obtained. Shires within a distance of 750 km were considered as neighbours with N_i defined as the set containing the neighbours of shire i .

Mathematically, the logarithm-of-odds ratio (OR) under the auto-binomial model is:

$$\ln\left(\frac{p(x_i, k_j)}{1 - p(x_i, k_j)}\right) = \alpha + \sum_{t=1}^8 x_t \beta_t + \gamma \sum_{j \in N_i} \frac{k_j}{d_{ij}},$$

where α is the overall intercept, β is the vector of coefficients associated with the covariates, γ is the coefficient of the 'spatial effect', and d_{ij} is the distance between shires i and j . This model reduces to the ordinary logistic regression model when γ is zero.

Estimation of the model parameters was done by using the maximum pseudo-likelihood approach (Johansson 2001), and the standard errors of the parameters were estimated by using delete-one jackknife resampling (Friedl and Stampfer 2002). The covariates in the final model were selected by using backward selection. Specifically, all covariates were included in the model at the beginning. After each iteration, the covariate with the greatest P -value was removed if the P -value was

>0.2. The process was repeated until all P -values were <0.2. Previous studies have shown that such a cut-off point would prevent elimination of some important covariates (Mickey and Greenland 1989; Maldonado and Greenland 1993).

Of the six herbicide groups considered for analysis, only the Group A dims showed a significant spatial effect in relation to incidence of resistance. Therefore, the resistance incidence of the other herbicide groups was analysed by using the ordinary logistic regression model. All estimations were carried out using R (R Core Team 2017). We refer readers to Ip *et al.* (2018) for further details of the statistical modelling.

Based on the final models obtained, the probability of each type of herbicide resistance was predicted for each shire. These predicted probabilities were then used to calculate the correlations. Based on how the indicator variables were introduced, a 'baseline' shire was a shire in Western Australia, predominantly alkaline, with crops other than wheat predominantly grown, predominantly with no cultivation, and crop residues predominantly managed other than left intact or incorporated.

Results

Note that some findings may appear significant, but the analysis indicates otherwise. This is because the statistical method considers more than one variable during the analysis. For example, differences between states may seem significant, but such differences result from different management practices rather than state *per se*.

The highest incidences of resistance were found to Groups A and B herbicides, with 79% of samples resistant to Group A fop herbicides (3360 tests), 23% to Group A dim herbicides (4738 tests, with some samples tested to more than one dim), 80% to Group A dens (188 tests), 57% to Group B SUs (3111 tests) and 69% to Group B imi herbicides (232 tests). Resistance incidences were much lower for the other main herbicide groups, with 7% resistant to Group D (3962 tests) and 3% to Group M (2279 tests). Resistance to Group C herbicides was <1% (3928 tests) (Table 3).

Correlations between herbicides

Significant correlations were observed between different herbicide groups and the incidence of resistance. Shires with a higher incidence of Group A fop resistance also had higher levels of Group A dim ($P < 0.001$), Group B ($P < 0.001$) and Group M ($P < 0.01$) resistance (Table 4). Increased Group A dim resistance was also correlated with increased Group B

resistance ($P<0.001$), and increased Group B resistance was correlated and increased Group M resistance ($P<0.001$). Group D resistance was negatively correlated with all other herbicide groups ($P<0.001$) except Group C, where a positive correlation ($P<0.01$) was reported (Table 4).

Table 3. Incidence of herbicide resistance (%) across six mode-of-action herbicide Groups (APVMA designation) in different states

Herbicide Group:	A fops	A dims	B	C	D	M
Australia	79.0	22.6	59.1	0.4	6.7	3.5
New South Wales	83.3	28.3	68.3	0.2	2.0	5.0
South Australia	68.7	13.7	32.4	0.2	15.2	0.0
Victoria	80.0	22.8	38.3	0.9	15.9	0.7
Western Australia	79.3	21.3	75.1	0.4	4.1	4.4

Table 4. Correlations between incidences of resistance for different mode-of-action herbicide Groups (APVMA designation)

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

	A dims	B	C	D	M
A fop'	0.60***	0.44***	-0.01	-0.38***	0.28**
A dim		0.41***	0.08	-0.35***	-0.07
B			-0.15	-0.94***	0.58***
C				0.26**	0.09
D					-0.64

Association between farm practice and herbicide resistance

Soil pH

Of the 121 shires across four states that fulfilled the criteria for analysis, 86 had soil types classed as acidic (71%) and 35 as alkaline (29%). Ten of the 'alkaline' shires were in South Australia (50% of South Australian shires that met the criteria), 17 in Western Australia (33%), six in Victoria (35%) and two in New South Wales (6%) (Fig. 1).

Compared with the base shire (alkaline), the odds of resistances evolving to Group A fop, Group A dim and Group B herbicides were estimated to be, respectively, 2.24 (95% confidence interval (CI) 1.86–2.69), 1.53 (95% CI 1.06–2.19) and 1.22 (95% CI 1.01–1.47) times higher in an 'acidic' shire (Table 5). No significant associations were found between soil pH and resistance to Groups C, D and M.

States

There were significant differences between states in the incidence of resistance to the different herbicide groups (Table 5) after taking into account all other variables. Compared with Western Australia, resistance to Group A fop herbicides was more likely in Victoria (OR 1.28, 95% CI 1.00–1.62) but less likely in South Australia (OR 0.59, 95% CI 0.47–0.73). Additionally, Group A fop resistance was found to be more likely in Victoria than South Australia (OR 2.17, 95% CI 1.67–2.83).

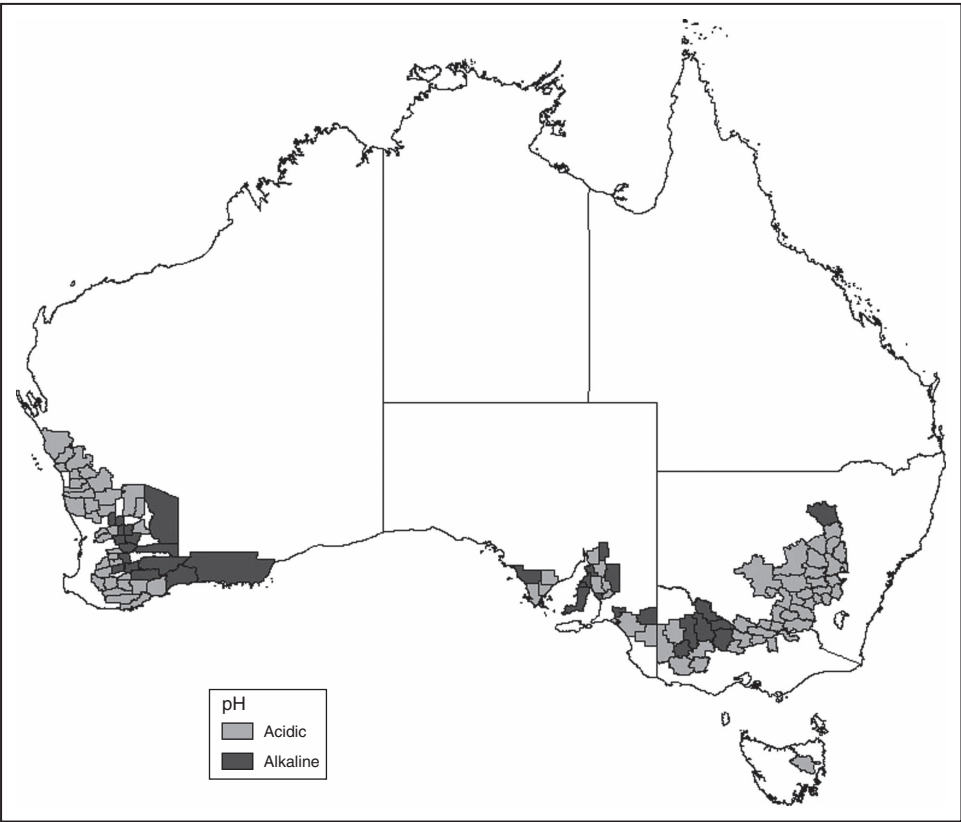


Fig. 1. Predominant soil pH classification, acidic or alkaline, for shires fitting the criteria for analysis.

Table 5. Estimates of the coefficients for the final model selected by backward selection
 * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Herbicide Groups are APVMA mode-of-action designations

	A fops	A dims	B	C	D	M
pH	0.806***	0.422*	0.0197*			
New South Wales		-1.491**	-2.236***		1.144	
Victoria	0.244*		-1.66***	1.092*	1.558***	-1.633*
South Australia	-0.532***		-1.74***		1.436***	-18.198
Wheat	0.179*	0.567*		-0.578*		-1.4526***
Cultivation		-0.457**	-0.214			
Intact		-1.338*	-1.820***		1.900**	-0.661*
Incorporated	-0.459*	-0.539	0.381*			
Spatial		0.181*				

Where results were available following data analysis, resistances to both Group A dim and B herbicides were more likely to occur in Western Australia, with the odds of Group A dim resistance in a New South Wales shire 0.23 (95% CI 0.08–0.67) times that of a Western Australian shire. The odds of Group B resistances in New South Wales, Victorian and South Australian shires were, respectively, 0.11 (95% CI 0.06–0.20), 0.19 (95% CI 0.15–0.24) and 0.18 (95% CI 0.14–0.23) times that in Western Australia (Table 5).

Resistance to Group D herbicides was, however, more likely in states other than Western Australia, with the odds of Group D resistance in Victoria and South Australia being, respectively, 4.75 (95% CI 3.41–6.62) and 4.20 (95% CI 3.02–5.85) times that in Western Australia (Table 5). Group M resistance was less likely to occur in Victoria than in Western Australia (OR 0.20, 95% CI 0.05–0.81).

Winter crop

Shires that grew more wheat were more likely to have resistance to Group A fop (OR 1.20, 95% CI 1.06–1.92) and Group A dim (OR 1.76, 95% CI 1.03–3.01) herbicides than shires growing a higher proportion of other crops. Conversely, shires that grew more wheat were less likely to have resistance to Group C (OR 0.56, 95% CI 0.10–0.99) and Group M (OR 0.23, 95% CI 0.13–0.42) herbicides than shires growing a higher percentage of other crops (Table 5).

Cultivation

Only for the Group A dim herbicides was a significant difference due to the number of cultivations identified after data analysis. The odds of Group A dim resistance in shires that predominantly have at least one cultivation was 0.63 (95% CI 0.45–0.89) times that in shires with predominantly no cultivation (Table 5).

Crop residue management

Compared with the baseline shire (crop residue managed other than by leaving intact or incorporating), the odds of resistance evolving to Group A dim, Group B and Group M were lower—0.26 (95% CI 0.09–0.78), 0.16 (95% CI 0.09–0.29) and 0.52 (95% CI 0.31–0.87) times, respectively—in those shires where the crop residue was more commonly left intact. Conversely the likelihood of Group D resistance was much higher (OR 6.69, 95% CI 1.80–24.8) where crop residue was left intact (Table 5). When increased proportions of crop residues were

incorporated, the likelihood of Group A fop resistance was reduced (OR 0.63, 95% CI 0.44–0.92) while resistance to the Group B herbicides was more likely (OR 1.46, 95% CI 1.00–2.14) than in shires using methods other than left intact or incorporation (Table 5).

Spatial effect

The spatial dependence parameter was found to be significantly positive for Group A dim resistance, meaning that incidences of dim resistance in neighbouring shires tended to be positively correlated.

Discussion

The significant correlation ($P < 0.001$) between Group A fop and dim herbicides reflects both their widespread use in winter crops and their control of annual ryegrass through inhibition of the same enzyme, acetyl-CoA carboxylase (Owen 1991). Group B herbicide resistance is also widespread and occurs over much of the same regions as for the Group A herbicides. This is shown by its positive association with both Group A fop and Group A dim herbicides, as was reported in an earlier study of New South Wales and Victorian regions (Broster and Pratley 2008).

The negative correlation between Group D and Group A fop, Group A dim and Group B herbicides is indicative of the differing application requirements. The Group D herbicides require soil incorporation immediately after application, whereas Group B herbicides are incorporated by sowing and Group A herbicides provide post-emergent, selective weed control. The Group D requirement for incorporation is largely incompatible with the direct-drilling system, whereas Groups A and B herbicides are routine options for direct drilling.

Resistances to Groups A and B herbicides were shown to be more likely where soils were acidic, whereas Group D resistance was more likely in the states with a high proportion of shires with alkaline soils (South Australia and Victoria) (Fig. 1, Tables 3 and 5). Both the Group A and Group B herbicides have high efficacy, which leads to their rapid uptake but also makes them prone to rapid development of herbicide resistance (Maxwell and Mortimer 1994; Saari *et al.* 1994). The pre-emergent Group B SU herbicides have limited incorporation requirement compared with many other pre-emergent herbicides, making them eminently suitable for minimum-tillage systems. However, the intensity of SU herbicide use in alkaline soils has historically been influenced by lengthy plant-back periods limiting rotational options, with

Group D herbicides a common alternative (Black *et al.* 1999; Boutsalis *et al.* 2006; Nufarm Australia 2018a). This has potentially resulted in the following: higher herbicide resistance in acidic soils due to reduced cultivation associated with Group B herbicide use; lower Group A resistance in alkaline soils because some weed control can be obtained by the cultivation needed for incorporation; and increased Group D resistance in the alkaline soils due to greater selection pressure (Boutsalis *et al.* 2006). As a consequence of high incidence of resistances to Group A and B herbicides, and a greater understanding of application techniques for minimum-tillage systems, the use of Group D herbicides may increase in acidic soils, meaning increased selection pressure for resistance to this herbicide group.

The associations of crop-residue management and herbicide-resistance incidence are more speculative, in that the analysis does not indicate whether the practice is linked directly with the establishment of the particular crop, management of the crop residue, management of the resistant weeds or some combination. The emphasis is likely to differ depending on the crop species and location.

The positive association between Group D resistance and retaining crop residue appears counter-intuitive. The major Group D herbicide used in Australia, trifluralin, is highly volatile and requires soil incorporation. It has low water solubility and may bind to any crop residue before reaching the soil surface (Chauhan *et al.* 2006; Borger *et al.* 2015; Lewis *et al.* 2016). The trifluralin label (for example, TriflurX 480 g a.i. L⁻¹) states that crop residue coverage >40–50% will limit weed control (Nufarm Australia 2018b), and this should limit its use where crop residue remains intact. However, reduced-tillage and crop-residue-retention systems have greater dependence on herbicides for weed control (D'Emden and Llewellyn 2006), and with increasing incidence of Groups A and B herbicide resistance (Table 3), Group D herbicides are used more often for pre-emergent ryegrass control because of the lack of alternative herbicide options. This has meant high selection pressure for resistance to Group D herbicides.

Shires growing a greater proportion of wheat had higher levels of resistance to Group A fop and dim herbicides. The lower risk of Group C resistance in these shires demonstrates the lower frequency of use of this group, limited to other crop species. This provides evidence of the importance of species diversity in crop rotations in enabling herbicide rotation, thereby reducing the likelihood of resistance by lowering selection pressure with respect to any herbicide groups commonly used in these rotations.

Many papers have investigated management practices for herbicide-resistant weeds (Powles and Matthews 1992; Gill 1996; Valverde *et al.* 2000; Beckie 2007; Moss *et al.* 2007; Walsh and Powles 2007; Norsworthy *et al.* 2012), and have also stated that there is a lack of weed control diversity before resistance developing (O'Connell and Allard 2004; Norsworthy *et al.* 2012). There is, however, limited knowledge of the influence of each of these management practices on resistance evolution (Beckie 2006). Beckie *et al.* (2004) reported that Group B resistance was higher in reduced-tillage systems, whereas Stanton *et al.* (2008) linked

the frequency of glyphosate use and amount of soil disturbance to the risk of resistance developing.

The findings of this study indicate the potential impact of farm practice on the evolution of herbicide resistance. Continued use of herbicides with the same mode of action predisposes the farm to an economic resistance problem. Cultural practices including the choice of crop species, soil pH, cultivation and crop residue retention influence the choice and frequency of use of herbicides and thus directly or indirectly influence resistance build-up over time. This study reinforces the principle of the need to rotate chemical modes of action to minimise the build-up of resistant weed populations.

Conflicts of interest

The authors declare no conflicts of interest.

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