



Towards large-scale prediction of *Lolium rigidum* emergence. II. correlation between dormancy and herbicide resistance levels suggests an impact of cropping systems

M J OWEN*†, P J MICHAEL‡, M RENTON*†§, K J STEADMAN¶ & S B POWLES*†

*Australian Herbicide Resistance Initiative, University of Western Australia, Crawley, WA, Australia, †School of Agriculture and Environment, Division of Science and Engineering, Curtin University of Technology, Northam, WA, Australia, ‡School of Plant Biology, University of Western Australia, Crawley, WA, Australia, §CSIRO Sustainable Ecosystems, Floreat WA, Australia, and ¶University of Queensland, School of Pharmacy and School of Biological Sciences, Brisbane, QLD, Australia

Received 7 May 2010

Revised version accepted 26 October 2010

Subject Editor: Adam Davis, USDA, USA

Summary

This study investigated a possible link between seed dormancy and herbicide resistance status of *Lolium rigidum* (annual or rigid ryegrass). Mature seeds were collected from 406 populations across the 14-million hectare grain belt of southern Western Australia. For each population, initial dormancy and change in dormancy over a 6-month period were measured, and resistance status of seedlings to four herbicides (diclofop-methyl, sethoxydim, clethodim and sulfometuron-methyl) was assessed. Greater seed dormancy correlated with higher levels of herbicide resistance for all four herbicides tested. The herbicides represented two modes of action (acetyl CoA carboxylase- and acetolactate

synthase inhibitors) and a contrast of generalist (metabolic) and target-site mutation mechanisms. The coexistence of dormancy and herbicide resistance is suggested to be an adaptation to decades of intense cropping; the plants that are most likely to successfully reproduce are those that exhibit delayed germination (avoiding pre-seeding weed control strategies) and possess herbicide resistance (surviving subsequent in-crop herbicide application). We propose that herbicide resistance status may have a role as a predictive tool in modelling dormancy in *L. rigidum* at a large spatial scale.

Keywords: annual ryegrass, survey, weed management, Mediterranean-type climate, seed dormancy, germination, herbicide resistance.

OWEN MJ, MICHAEL PJ, RENTON M, STEADMAN KJ & POWLES SB (2010). Towards large-scale prediction of *Lolium rigidum* emergence II: correlation between dormancy and herbicide resistance levels suggests an impact of cropping systems. *Weed Research*.

Introduction

Lolium rigidum Gaud. (annual or rigid ryegrass) is a genetically variable cross-pollinated species that is widespread across grain-growing regions of Australia. This winter-germinating annual grass weed is often present at high densities and has evolved resistance to

many herbicides (Llewellyn & Powles, 2001; Owen *et al.*, 2007). For decades, herbicides have been the primary means of controlling *L. rigidum* in field crops. Consequently, continual selection pressure from the use of herbicides has resulted in the evolution of populations resistant to both selective and non-selective herbicides across at least nine different herbicide

mode-of-action groups (Burnet *et al.*, 1994; Preston & Powles, 2002).

Long-term intensive crop production systems that are based on reduced tillage and stubble retention are common in the Western Australian (WA) grain belt (D'Emden *et al.*, 2006) and are highly reliant on herbicide applications for weed control (Beckie, 2006). Weed control strategies are regularly applied at two different points in time during the winter-cropping system. First, a non-selective control event prior to seeding (either herbicide or mechanical) eliminates weed seedlings arising from less dormant seeds; then, an in-crop, post-emergent selective herbicide is applied to those weed seedlings which germinate after the crop is sown and therefore represent the more dormant portion of the weed seed population. This farming practice, when used annually, results in a strong selection pressure for herbicide resistance in weed seedlings emerging after the crop is planted. For the in-crop selective herbicides, resistance can develop in as few as 4 years (Powles *et al.*, 1997; Preston *et al.*, 1999). Herbicide-resistant weed evolution has led to the need for more integrated weed management practices for *L. rigidum* control, which requires detailed knowledge of *L. rigidum* biology, particularly the timing of seedling emergence. With a long-term goal of predicting the timing and extent of weed emergence across a large area, we have previously investigated the potential to use climatic parameters as a predictor, because temperature and rainfall are known to be important modulators of seed dormancy and germination in *L. rigidum*. However, we concluded that predictive power at the scale of the WA grain belt was limited when based on the use of climatic parameters alone (Owen *et al.*, 2010).

Suggestions have occasionally been made in the literature of a link between herbicide resistance and seed dormancy. Of particular relevance to this study is the measurement of differences in seed germination and emergence responses between herbicide-susceptible and herbicide-resistant individuals (Vila-Aiub *et al.*, 2005) and populations of *L. rigidum* (Gill *et al.*, 1996; Recasens *et al.*, 2007). Gill *et al.* (1996) found only a tenuous link between seed dormancy status and herbicide resistance in 28 populations collected from various locations in the WA grain belt; a later study by Vila-Aiub *et al.* (2005) used plants that were all derived from a single, multiple-resistant population originating from one field. The more dormant seeds tended to produce individuals exhibiting resistance because of a target-site acetyl CoA carboxylase (ACCase) mutation, whereas individuals from the less dormant seeds exhibited either herbicide susceptibility or resistance because of a non-target-site cytochrome P450-based enhanced metabolism mechanism (Vila-Aiub *et al.*, 2005). This link is not

restricted to *L. rigidum*; resistant biotypes have been associated with greater dormancy than susceptible biotypes in a number of other weed species. For example, *Amaranthus retroflexus* L. and *Amaranthus powellii* S. Wats. biotypes exhibiting triazine resistance had lower and slower germination, attributed to deeper dormancy, than susceptible biotypes (Weaver & Thomas, 1986). Fewer germinating seeds were observed for triazine-resistant *Chenopodium album* L. and *Solanum nigrum* L. (Gasquez *et al.*, 1981), slower germination for triazine-resistant *Brassica campestris* L. (Mapplebeck *et al.*, 1982), lower germination in triazine-resistant *Setaria faberi* Herrm. (Tranel & Dekker, 2002) and delayed emergence for a diclofop-methyl-resistant population of *Lolium multiflorum* Lam. (Ghersa *et al.*, 1994). Conversely, several studies have reported lower dormancy in herbicide-resistant biotypes; for example, higher germination of trillate/diflufenican-resistant *Avena fatua* L. (O'Donovan *et al.*, 1999) and diclofop-resistant *A. fatua* (Scursoni *et al.*, 1999), earlier, faster and greater germination of triazine-resistant *S. nigrum* L. (Kremer & Lotz, 1998) and more rapid germination in sulfonylurea-resistant *Lactuca serriola* L. (Alcocer-Ruthling *et al.*, 1992).

In this study, we considered whether there was any association between herbicide resistance and seed dormancy in *L. rigidum* that could lead to resistance status being a potential predictor for the timing of seed germination. This was achieved by assessing 406 populations collected from across the 14-million hectare crop production region of southern Western Australia. This study is the second paper in a series, in which the first examined the ability to predict seed dormancy in *L. rigidum* based on climatic parameters over a large spatial scale, such as the WA grain belt (Owen *et al.*, 2010). This study is the first stage of assessing the association between herbicide resistance status and dormancy status. We hypothesised that (i) herbicide resistance and dormancy are associated and (ii) this association would be strong enough that herbicide resistance level would be a useful predictor of dormancy in *L. rigidum*.

Materials and methods

Seed collection

Crop fields were surveyed prior to the 2003 grain harvest, with *L. rigidum* seeds collected over a period of 4 weeks, November to December 2003. In total, 500 cropping fields were visited across 15 agronomic zones in the WA grain belt (see Fig. 1 in Owen *et al.*, 2010). For more detailed sample collection methods, see Owen *et al.* (2007). All samples were stored in paper bags in a ventilated glasshouse for 2–3 weeks before being moved

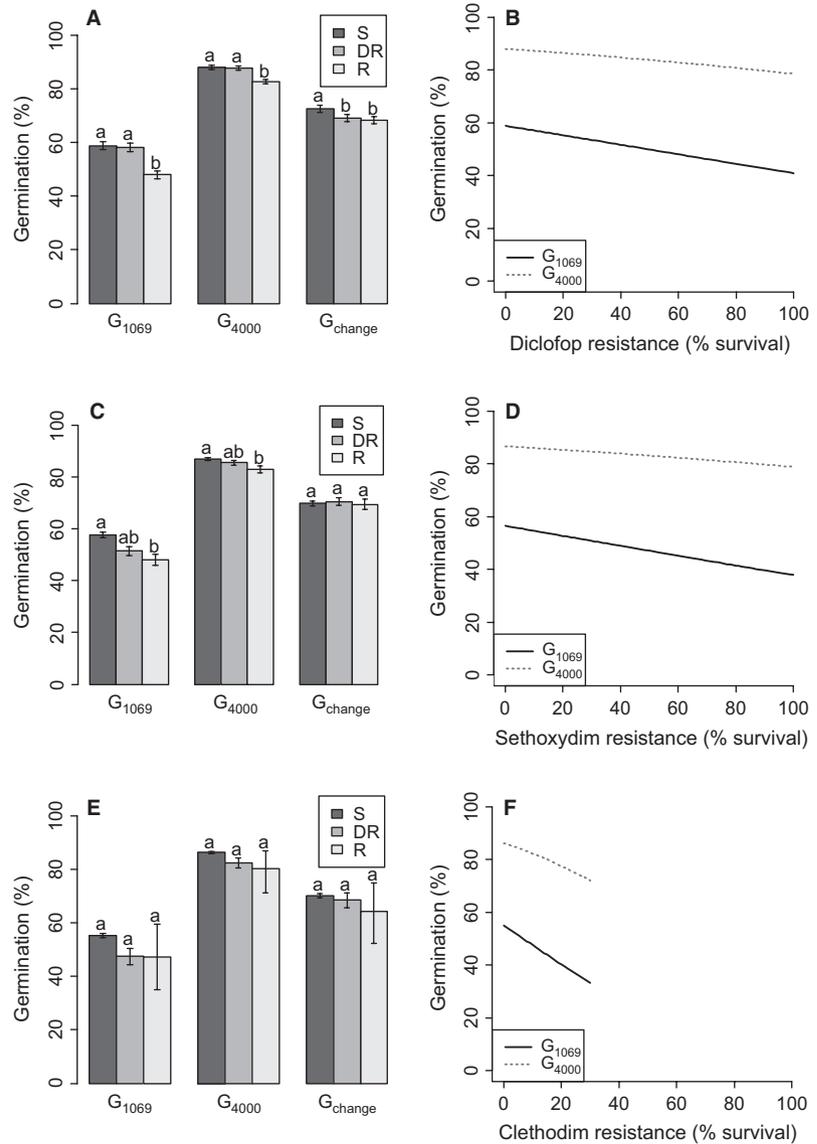


Fig. 1 The relationship between dormancy characteristics and resistance category (A, C, E) or percentage (B, D, F) to diclofop (A, B), sethoxydim (C, D) and clethodim (E, F). When assessed in terms of herbicide resistance category, the height of the shaded bar represents the average \pm SE for dormancy parameters, as estimated by the fitted generalised linear model (GLM). Letters above the bars that differ from each other indicate the values are significantly different ($P < 0.05$) according to the fitted GLM and to Tukey's honest significant difference test. Resistance categories were classed as resistant (R; $>20\%$ survival), developing resistance (DR; $1\text{--}19\%$ survival) and susceptible (S; $<1\%$ survival). When assessed in terms of herbicide resistance on a continuous scale, the lines represent the mean for dormancy parameters as modelled by a quasibinomial GLM. No populations had $>26\%$ clethodim resistance (F).

to the laboratory at 23°C for 1 week, during which time seeds were threshed and separated from the chaff by sieving and forced-air separation. Seed populations with <2 g (c. 850 seeds) of clean seed were excluded from this study. Two grams of seed was considered adequate to assess dormancy status, dormancy release and herbicide resistance, and there were 406 of the 452 populations collected that met this criterion.

After-ripening and germination testing

Freshly harvested seeds were placed in paper bags and stored in a dry ventilated glasshouse (45–55% relative humidity from December 2003 to May 2004) for after-ripening; air temperature was logged using a Tiny Tag Transit data logger (Gemini, Chichester, UK) located with the bags. Average temperatures and humidity experienced in the glasshouse during the after-ripening

period were similar to field conditions, with average daily temperatures ranging from 22 to 28°C over the summer period. Change in seed dormancy during after-ripening was assessed at monthly intervals (7 months in total) on 50 seeds from each of the 406 populations, with germination testing on 1% (w/v) solidified agar–water at $25/15^{\circ}\text{C}$ with a 12-h photoperiod. The number of germinated seeds were counted after 28 days, with the criterion for germination being visible radicle protrusion. Viability of all populations was $>95\%$ (tested by gently squeezing the seed and with empty seeds excluded at the time of set-up). Detailed germination testing is described in Owen *et al.*, 2010.

Herbicide resistance testing

Herbicide resistance status was determined by treating 2- to 3-leaf stage seedlings with four herbicides, using the

upper recommended rate and assessing plant mortality 21 days after treatment (see Owen *et al.*, 2007 for detailed methodology). Fifty seedlings from each population were treated with each herbicide during the 2004 growing season (May–September). Herbicides were applied using a custom built, dual nozzle (TeeJet® XR11001 flat fan) cabinet sprayer delivering herbicide in 110 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; BayerCropScience, Australia) and clethodim (Select 240 g a.i L⁻¹; Sumitomo Chemical, Australia), and the acetolactate synthase (ALS)-inhibiting herbicide sulfometuron-methyl (Oust 750 g a.i. kg⁻¹; DuPont, Australia). The rates used for diclofop-methyl, sethoxydim, clethodim and sulfometuron-methyl were 563, 186, 60 and 15 g a.i. ha⁻¹ respectively (Table 1), which are the upper recommended commercial field rates for *L. rigidum* in Australia. Known susceptible and resistant controls were used in all experiments with 100% control of the known susceptible population and high survival (>90%) of the known resistant populations (data not shown).

Resistance to ACCase inhibitor herbicides may be because of enhanced rates of metabolism (Preston *et al.*, 1996; Preston & Powles, 1998) and/or different ACCase mutations (Tardif & Powles, 1994; Zhang & Powles, 2006a,b). Individuals that survived the application of diclofop-methyl were subsequently treated with sethoxydim, as resistance to sethoxydim can be an indicator of an ACCase mutation (Tardif & Powles, 1994). Individuals that are resistant to the ALS-inhibiting herbicide sulfometuron-methyl most likely contain a resistance-endowing mutation in the ALS enzyme (Preston & Powles, 2002). For each herbicide, the resistance status of the populations was considered in two ways: as the proportion of the population able to survive the herbicide application (a continuous variable) and as classifications of susceptible, developing resistance

Table 1 Number of populations in each resistance category, the mean % level of resistance within those categories and the herbicide rate used

Herbicide	Rate (g a.i.)	Resistance Class (R, DR & S) and mean % resistance					
		R	%	DR	%	S	%
Diclofop-methyl	563	151	57.2	122	7.7	133	0
Sethoxydim	186	141	60.7	27	11.8	238	0
Clethodim	60	2	24.1	32	5.8	372	0
Sulfometuron-methyl	15	271	69.9	83	8.5	52	0

Categories were classed as resistant (R; >20% survival), developing resistance (DR; 1–19% survival) and susceptible (S; <1% survival).

or resistant (discrete categories). Populations were classed as resistant if 20% or more of the individuals in the population survived the herbicide, as developing resistance if 1–19% survived, and as susceptible if there was less than 1% survival. These categories are consistent with previous resistance surveys by Llewellyn and Powles (2001) and Owen *et al.* (2007) and are maintained in this study as this is a management-relevant system of classification. At the field management level, farmers often recognise resistance at a level of around 20% survival and may stop using the herbicide at that point.

Statistical analysis

Logistic regression was used to model release of dormancy as a function of after-ripening as described in the first paper of this series, Owen *et al.* (2010). The temperature experienced during after-ripening was converted to thermal time (Steadman *et al.*, 2003), the cumulative day degrees from seed collection (when seeds had reached maturity; a time that was similar for all populations). Separate three-parameter logistic growth curve models for germination were fitted for each population, which led to a total of 406 logistic models of the form:

$$G_i = \frac{G_{\max}}{1 + \exp(b + DRR(t_i - t_0))} \quad (1)$$

The three parameters G_{\max} , dormancy release rate (DRR) and b were thus estimated separately for each of the 406 populations, using the function *nlm* in the R programming language (R Development Core Team, 2007) to minimise negative log-likelihood (detailed in Owen *et al.*, 2010). The parameter G_{\max} represents the upper bound on the per cent of seeds losing dormancy as thermal time becomes large (the asymptote) and is constrained to be ≤ 100 , the slope parameter DRR describes the dormancy release rate and b is a 'horizontal shift' parameter closely related to the germination per cent at t_0 (Steadman *et al.*, 2004). For each population, the parameterised version of Eqn 1 was then used to obtain estimates for three further parameters describing the dormancy status of the population; G_{1069} (germination 1069 day degrees (d °C) after collection), G_{4000} (germination 4000 day degrees after collection) and G_{change} . The third parameter, G_{change} , was recorded as a measure of the relative change in germinability from summer to winter, where G_{change} is defined by

$$G_{\text{change}} = \frac{G_{4000} - G_{1069}}{100 - G_{1069}} \quad (2)$$

and thus represents the percentage of seeds that were dormant at 1069 d °C, but had lost dormancy by

4000 d °C. The first parameter G_{1069} was chosen as it was a time when all populations had been tested and is a measure of population germinability in summer, at the beginning of after-ripening. The second parameter G_{4000} was the latest time at which all populations were still to have a final germination test and was a measure of population germinability in winter (coinciding with crop planting). To represent the dormancy characteristics of each population, we chose to use the three calculated parameters, G_{1069} , G_{4000} and G_{change} , in addition to DRR as a fourth parameter that describes the speed of release of seed dormancy for each population.

A 'derived variable analysis' was conducted to examine relationships between the four dormancy status parameters and herbicide resistance status, with the latter considered as both a continuous variable and as a discrete categorical classification, as described above. Derived variable analysis, which involves reducing repeated measures into a set of summary statistics (G_{1069} , G_{4000} and G_{change} and DRR in this case) and then analysing them using standard parametric techniques, is a valid method for avoiding pseudo-replication (Crawley, 2007). When considering the ability of the herbicide resistance measures to predict each of the dormancy parameters (G_{1069} , G_{4000} and G_{change}), we fitted a generalised linear model (GLM) with a logistic link function and quasibinomial errors. When considering the ability of the herbicide resistance measures to predict the DRR, a negative log-transformation was made (which normalised errors) and then a linear model was fitted against the transformed variable $-\log(-DRR)$. Data presented are transformed for the DRR parameter (so higher values correspond to faster dormancy release) and untransformed for the dormancy parameters. For the GLM, an analysis of deviance was performed to determine significance using the R program function `anova.glm` and the Chi-squared test, while for the linear model a normal ANOVA was used. Both tests are referred to as ANOVA below, and all tests were conducted at the $P = 0.05$ significance level, unless stated otherwise. All statistical analysis was carried out in the R programming language (R Development Core Team, 2007).

Results

For all 406 *L. rigidum* populations collected at maturity from crop fields across the WA grain belt, increasing proportions of seeds were able to germinate as after-ripening progressed, although the way that seed germinability changed with increasing thermal after-ripening time was variable (Owen *et al.*, 2010). For all herbicides, higher levels of resistance in the population were associated with lower levels of germination.

The relationship was relatively weak but statistically significant and showed similar trends for all herbicides, with the exception of clethodim, which had only a small number of resistant populations with low levels of resistance; the overall level of resistance for diclofop-methyl, sethoxydim and sulfometuron-methyl was >57%, whereas clethodim had 24% resistance (Table 1).

Resistance to all four herbicides tested, whether measured as percentage survival (continuous classes) or as resistance categories (discrete classes), exhibited a significant correlation with dormancy status, whether measured as summer (G_{1069}) or winter (G_{4000}) germination, except for clethodim in discrete classes and G_{4000} (Table 2). In all cases, an increased level of herbicide resistance was associated with a lower level of seed germination, i.e. greater dormancy. Change in germination (G_{change}) was only linked significantly to sulfometuron-methyl resistance, and DRR had no significant association with resistance status (Table 2).

Populations classed as resistant to diclofop-methyl had significantly lower summer and winter germination than those populations that were susceptible or developing resistance (Fig. 1A). When predicting dormancy status from the percentage survival of plants in a population, the same significant correlations were observed as with the resistance categories (Table 2), with increasing levels of dormancy again associated with increasing levels of herbicide resistance (Fig. 1B). When diclofop-resistant individuals for each population were subsequently treated with sethoxydim, once again high levels of resistance were associated with higher levels of dormancy for both the continuous and discrete variable measures (Fig. 1C, D).

Only two populations of the 406 tested had clethodim resistance (Table 1); thus, there is a limited ability to draw conclusions from these data. However, taking into account both the treatment of the data as discrete categories (Fig. 1E) and continuous variables (Fig. 1F), there is certainly some indication of lower germination associated with higher herbicide resistance, even with the small quantity of data (Table 2). Notably, the clethodim-resistant populations occurred to a greater extent in the northern agricultural zone M2 (Owen *et al.*, 2007); this is also the agricultural zone containing populations exhibiting greater dormancy (Owen *et al.*, 2010).

Sulfometuron-methyl resistance status was also a particularly strong predictor for reduced germination because of dormancy, whether measured in summer or winter (Fig. 2A, Table 2) and the relationship was very clear using percentage survival, with higher herbicide resistance being associated with lower germination and smaller change in germination between summer and winter (Fig. 2B, Table 2).

	G_{1069}	G_{4000}	G_{change}	$-\log(-\text{DRR})$
Continuous variable				
<i>Herbicide resistance level</i>				
Diclofop	<0.001 (9%)	<0.001 (8%)	n.s.	n.s.
Sethoxydim	<0.001 (4%)	0.004 (2%)	n.s.	n.s.
Clethodim	0.033 (1%)	0.036 (1%)	n.s.	n.s.
Sulfometuron-methyl	<0.001 (9%)	<0.001 (10%)	<0.001 (5%)	n.s.
Discrete variable				
<i>Herbicide resistance category</i>				
Diclofop	<0.001	<0.001	n.s.	n.s.
Sethoxydim	<0.001	0.012	n.s.	n.s.
Clethodim	0.044	n.s.	n.s.	n.s.
Sulfometuron-methyl	<0.001	<0.001	<0.006	n.s.

For significant results ($P < 0.05$) for the continuous variable measure, the relationship was negative in all cases (greater resistance associated with lower germinability) and the percentage of deviance explained by the model is given in parentheses. GLM, generalised linear model.

Note that the average germination for each of the resistance categories is shown in bar plots and the way that the average germination changes with the continuous resistance data is shown in the line plots in Fig. 1. The plots show that the differences in germination (the effect size) between resistance categories and levels are small, but are still biologically and agronomically meaningful, as well as being statistically significant. For example, summer germination varies from *c.* 60% to *c.* 40% as diclofop resistance varies from 0% to 100% (Fig. 1). The proportions of deviance explained by the GLM model for the continuous data are presented in Table 2. The amount of deviance explained is small, ranging from 1% to 10%.

Discussion

This study has demonstrated that greater seed dormancy in *L. rigidum* is correlated with higher levels of herbicide

Table 2 Significance of relationships between continuous variable and discrete variable measures for herbicide resistance level and dormancy status parameters (G_{1069} , summer germination; G_{4000} , winter germination; G_{change} , change in germination between summer and winter; DRR, dormancy release rate) as modelled by a quasibinomial GLM with logistic link function

resistance for four different herbicides representing two modes of action and a contrast of generalist (metabolic) and target-site mutation mechanisms. This supports our hypothesis that dormancy and herbicide resistance are correlated. As suggested by Gundel *et al.* (2008), it is unlikely that the direct pleiotropic effect of resistant alleles, or even linkage of germination-influencing alleles to resistance alleles, is the reason for this correlation between germination and resistance. It is also unlikely that populations that survive herbicide application subsequently become dormant. We believe this association is likely to be caused by management factors. We explain this coexistence of deeper seed dormancy and herbicide resistance in terms of the selection pressure applied by decades of intense cropping in certain regions of the grain belt in Western Australia. The region relies on weed control strategies that are applied as a non-selective control event prior to seeding and then as an in-crop, post-emergent selective herbicide during the

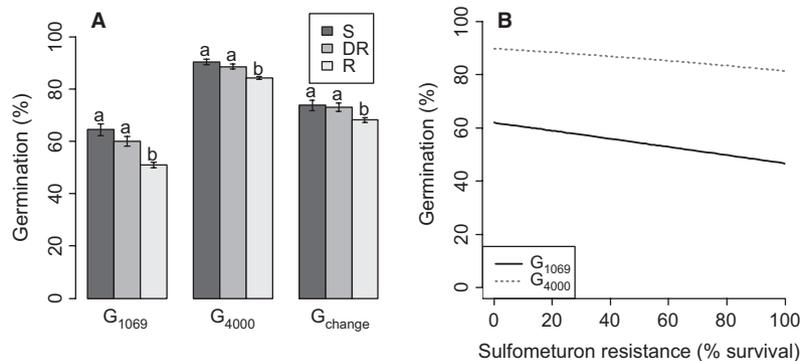


Fig. 2 The relationship between dormancy characteristics and resistance category (A) or percentage (B) to the acetolactate synthase inhibitor herbicide sulfometuron-methyl. When assessed in terms of herbicide resistance category, the height of the shaded bar represents the average \pm SE for dormancy parameters, as estimated by the fitted generalised linear model (GLM). Letters above the bars that differ from each other indicate the values are significantly different ($P < 0.05$) according to the fitted GLM and to Tukey's honest significant difference test. Resistance categories were classed as resistant (R; >20% survival), developing resistance (DR; 1–19% survival) and susceptible (S; <1% survival). When assessed in terms of herbicide resistance on a continuous scale, the lines represent the mean for dormancy parameters as modelled by a quasibinomial GLM.

winter cropping season. Consequently, it is the plants that combine dormancy and resistance to selective herbicides that successfully reproduce and contribute to the seedbank.

Long-term intensive crop production systems are utilised in the WA grain belt (D'Emden *et al.*, 2006) with pre-seeding and in-crop weed control strategies being common features of southern Australian winter cropping systems, both being applied within any year that a field is cropped. The least dormant seeds of a *L. rigidum* population lose dormancy during the summer and are able to germinate and emerge as soon as consistent rainfall breaks the summer drought. In cropping years, these non-dormant seedlings are removed by a highly effective (95%) pre-seeding weed control event known as a 'knock-down', involving either a shallow cultivation or non-selective herbicide, such as glyphosate or paraquat (Neve *et al.*, 2003b). Non-selective herbicides are categorised as having a low likelihood of causing the development of resistance (reviewed in Preston *et al.*, 1999; Neve *et al.*, 2003a,b) because the resistance gene is rare in *L. rigidum* populations (Neve *et al.*, 2003a); and therefore, it would be less likely that these early germinating seeds would be resistant to these non-selective herbicides. Therefore, a mechanism (such as dormancy) that allows weeds to avoid non-selective herbicides is more likely to be selected for than resistance to these herbicides. In contrast to the non-selective herbicides, resistance can develop very quickly (after 3–5 applications) for the post-emergent in-crop selective herbicides (Powles *et al.*, 1997; Preston *et al.*, 1999). Avoiding selective herbicides by delaying germination until after the crop is established is likely to result in severe competitive disadvantages. Therefore, a level of dormancy that allows weeds to avoid selective herbicides is less likely to be selected for than resistance to these herbicides.

Herbicide application may result in directional selection for traits other than resistance. As explained by Gundel *et al.* (2008), it is unlikely that deeper dormancy is caused by the presence of genes responsible for herbicide resistance, but instead that herbicide application has acted as a selection pressure for phenological adaptation of traits affecting germination behaviour. In a 5-year long-field trial with *L. multiflorum*, seed behaviour traits such as dormancy were modified by herbicide selection history, but dormancy release was not directly related to the level of herbicide resistance (Gundel *et al.*, 2008). We believe that the selection for seed dormancy observed for *L. rigidum* is a phenological adaptation for late seedling emergence to avoid pre-seeding weed control (Mortimer, 1997; Gundel *et al.*, 2008). Herbicide application may also select for seed traits such as delayed germination (Sbatella & Wilson, 2010). Our

results show that the populations with the greater percentage of resistant individuals are also the populations with the greatest percentage of dormant seeds. This could suggest that these more dormant seeds have adapted to escape unfavourable environmental conditions; however, delayed germination then exposes seedlings to post-emergent herbicide application.

Agronomic practices such as cultivation can also act as a selection pressure for dormancy in many weeds (Jana & Thai, 1987; Murphy & Lemerle, 2006; Brainard *et al.*, 2007). Individual management practices may have long-term implications on seed characteristics such as dormancy and timing of emergence. A study that examined different crop rotation effects on *A. powellii* seed characteristics found lower germination among seeds originating from dairy crop rotations when compared with vegetable farms with a long cropping history (rotations 10 years or longer) (Brainard *et al.*, 2007). Brainard *et al.* (2007) suggest that multiple years of perennial lucerne crops (3–5 years) in the dairy rotations may exert selection pressure for greater seed dormancy in the annual weed *A. powellii*, as they cannot mature successfully to reproduce in established lucerne. Jana and Thai (1987) also reported that summer fallow systems selected for greater seed dormancy in *A. fatua* and proposed that *A. fatua* populations continuously exposed to herbicides are more likely to contain a larger frequency of dormant individuals. Our results support this research with evidence that the more resistant *L. rigidum* populations contain a larger proportion of dormant seed.

Our results only partly support the hypothesis that herbicide resistance may be a useful predictor of dormancy in *L. rigidum*. The proportion of deviance explained by the model shows that resistance status explains only a relatively small portion of total variability in germination, and the results also show that the difference in average germination between the resistant and susceptible populations is small (10–20%) but still significant. Resistance status can give a small, yet significant and useful amount of predictive ability regarding dormancy levels in a field. For example, growers with high levels of resistance could expect to have more dormant *L. rigidum* populations, which would germinate later in the growing season. As herbicide resistance is associated with intensive cropping history, in the absence of further information, growers could delay seeding in fields with a long cropping history and apply alternative weed control practices.

Our results show a significant association between herbicide resistance and seed dormancy in *L. rigidum* over a large spatial scale. Further research is underway to understand the causes of this relationship, with the ultimate goal of developing more robust predictive

models. This work also provides further evidence for the need to consider the implications of applying the same crop management practices year-after-year on weed population dynamics and resistance evolution.

Acknowledgements

This project was funded by the Grains Research and Development Corporation. We thank the members of the AHRI research team (staff and students) who assisted in many areas of the project.

References

- ALCOCKER-RUTHLING M, THILL DC & SHAFII B (1992) Seed biology of sulfonylurea-resistant and -susceptible biotypes of prickly lettuce (*Lactuca serriola*). *Weed Technology* **6**, 858–864.
- BECKIE HJ (2006) Herbicide-resistant weeds: management tactics and practices. *Weed Technology* **20**, 793–814.
- BRAINARD DC, DiTOMMASO A & MOHLER CL (2007) Intra-specific variation in seed characteristics of powell amaranth (*Amaranthus powellii*) from habitats with contrasting crop rotation histories. *Weed Science* **55**, 218–226.
- BURNET MWM, CHRISTOPHER JT, HOLTUM JAM & POWLES SB (1994) Identification of two mechanisms of sulfonylurea resistance within one population of rigid ryegrass (*Lolium rigidum*) using a selective germination medium. *Weed Science* **42**, 468–473.
- CRAWLEY MJ (2007) *The R Book*. John Wiley and Sons, Chichester, UK.
- D'EMDEN FH, LLEWELLYN RS & BURTON MP (2006) Adoption of conservation tillage in Australian cropping regions: an application of duration analysis. *Technological Forecasting and Social Change* **73**, 630–647.
- GASQUEZ J, DARMENCY H & COMPOINT JP (1981) Comparaison de la germination et de la croissance de biotypes sensibles et résistants aux triazines chez quatre espèces de mauvaises herbes. *Weed Research* **21**, 219–225.
- GHERSA CM, MARTINEZ-GHERSA MA, BREWER TG & ROUSH ML (1994) Selection pressures for diclofop-methyl resistance and germination time of Italian ryegrass. *Agronomy Journal* **86**, 823–828.
- GILL GS, COUSENS RD & ALLAN MR (1996) Germination, growth and development of herbicide resistant and susceptible populations of rigid ryegrass (*Lolium rigidum*). *Weed Science* **44**, 252–256.
- GUNDEL PE, MARTINEZ-GHERSA MA & GHERSA CM (2008) Dormancy, germination and ageing of *Lolium multiflorum* seeds following contrasting herbicide selection regimes. *European Journal of Agronomy* **28**, 606–613.
- JANA S & THAI KM (1987) Patterns of changes of dormant genotypes in *Avena fatua* populations under different agricultural conditions. *Canadian Journal of Botany* **65**, 1741–1745.
- KREMER E & LOTZ LAP (1998) Germination and emergence characteristics of triazine-susceptible and triazine-resistant biotypes of *Solanum nigrum*. *Journal of Applied Ecology* **35**, 302–310.
- LLEWELLYN RS & POWLES SB (2001) High levels of herbicide resistance in rigid ryegrass (*Lolium rigidum*) in the wheat belt of Western Australia. *Weed Technology* **15**, 242–248.
- MAPPLEBECK LR, SOUZA MACHADO V & GRODZINSKI B (1982) Seed germination and seedling growth characteristics of atrazine susceptible and resistant biotypes of *Brassica campestris*. *Canadian Journal of Plant Science* **62**, 733–739.
- MORTIMER AM (1997) Phenological adaptation in weeds – an evolutionary response to the use of herbicides? *Pesticide Science* **51**, 299–304.
- MURPHY CE & LEMERLE D (2006) Continuous cropping systems and weed selection. *Euphytica* **148**, 61–73.
- NEVE P, DIGGLE AJ, SMITH FP & POWLES SB (2003a) Simulating evolution of glyphosate resistance in *Lolium rigidum* I: population biology of a rare resistance trait. *Weed Research* **43**, 404–417.
- NEVE P, DIGGLE AJ, SMITH FP & POWLES SB (2003b) Simulating evolution of glyphosate resistance in *Lolium rigidum* II: past, present and future glyphosate use in Australian cropping. *Weed Research* **43**, 418–427.
- O'DONOVAN JT, NEWMAN JC, BLACKSHAW RE, HARKER KN, DERKSEN DA & THOMAS AG (1999) Growth, competitiveness and seed germination of triallate/difenoquat-susceptible and -resistant wild oat populations. *Canadian Journal of Plant Science* **79**, 303–312.
- OWEN MJ, WALSH MJ, LLEWELLYN RS & POWLES SB (2007) Widespread occurrence of multiple herbicide resistance in Western Australian annual ryegrass (*Lolium rigidum*) populations. *Australian Journal of Agricultural Research* **58**, 711–718.
- OWEN MJ, MICHAEL PJ, RENTON M, STEADMAN KJ & POWLES SB (2010) Towards large-scale prediction of *Lolium rigidum* emergence. I: can climate be used to predict dormancy parameters? *Weed Research*, DOI: 10.1111/j.1365-3180.2010.00832.x.
- POWLES SB, PRESTON C, BRYAN IB & JUTSUM AR (1997) Herbicide resistance: impact and management. *Advances in Agronomy* **58**, 57–93.
- PRESTON C & POWLES SB (1998) Amitrole inhibits diclofop metabolism and synergises diclofop-methyl in a diclofop-methyl-resistant biotype of *Lolium rigidum*. *Pesticide Biochemistry and Physiology* **62**, 179–189.
- PRESTON C & POWLES SB (2002) Evolution of herbicide resistance in weeds: initial frequency of target site-based resistance to acetolactate synthase-inhibiting herbicides in *Lolium rigidum*. *Heredity* **88**, 8–13.
- PRESTON C, TARDIF FJ, CHRISTOPHER JT & POWLES SB (1996) Multiple resistance to dissimilar herbicide chemistries in a biotype of *Lolium rigidum* due to enhanced activity of several herbicide degrading enzymes. *Pesticide Biochemistry and Physiology* **54**, 123–134.
- PRESTON C, ROUSH RT & POWLES SB (1999) Herbicide resistance in weeds of southern Australia: why are we the worst in the world? In: *12th Australian Weeds Conference: Papers and Proceeds* (eds AC BISHOP, M BOERSMA & CD BARNES), 454–459. Tasmanian Weed Society, Davenport, Australia.
- R DEVELOPMENT CORE TEAM (2007) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>.

- RECASENS J, CAIMONS O, TORRA J & TABERNER A (2007) Variation in seed germination and early growth between and within acetolactate synthase herbicide resistant and susceptible *Lolium rigidum* accessions. *Seed Science and Technology* **35**, 32–47.
- SBATELLA GM & WILSON RG (2010) Isoxaflutole shifts *Kochia* (*Kochia scoparia*) populations in continuous corn. *Weed Technology* **24**, 392–396.
- SCURSONI JA, BENECH-ARNOLD RL & HIRCHOREN H (1999) Demography of wild oat (*Avena fatua* L.) in barley crops; effect of crop, sowing rate and herbicide treatment. *Agronomy Journal* **91**, 478–485.
- STEADMAN KJ, CRAWFORD AJ & GALLAGHER RS (2003) Dormancy release in *Lolium rigidum* seeds is a function of thermal after-ripening time and seed water content. *Functional Plant Biology* **30**, 345–352.
- STEADMAN KJ, ELLERY AJ, CHAPMAN R, MOORE A & TURNER NC (2004) Maturation temperature and rainfall influence seed dormancy characteristics of annual ryegrass (*Lolium rigidum*). *Australian Journal of Agricultural Research* **55**, 1047–1057.
- TARDIF FJ & POWLES SB (1994) Herbicide multiple-resistance in a *Lolium rigidum* biotype is endowed by multiple mechanisms: isolation of a subset with resistant acetyl-CoA carboxylase. *Physiologia Plantarum* **91**, 488–494.
- TRANEL DM & DEKKER J (2002) Differential seed germinability in triazine-resistant and -susceptible giant foxtail (*Setaria faberii*). *Asian Journal of Plant Science* **1**, 334–336.
- VILA-AIUB MM, NEVE P, STEADMAN KJ & POWLES SB (2005) Ecological fitness of a multiple herbicide resistant *Lolium rigidum* population: dynamics of seed germination and seedling emergence of resistant and susceptible phenotypes. *Journal of Applied Ecology* **42**, 288–298.
- WEAVER SE & THOMAS AG (1986) Germination response to temperatures of atrazine-resistant and susceptible biotypes of two pigweed (*Amaranthus*) species. *Weed Science* **34**, 865–870.
- ZHANG X & POWLES SB (2006a) The molecular basis for resistance to acetyl co-enzyme A carboxylase (ACCase) inhibiting herbicides in two target-based resistant biotypes of annual ryegrass (*Lolium rigidum*). *Planta* **223**, 550–557.
- ZHANG X & POWLES SB (2006b) Six amino acid substitutions in the carboxyl-transferase domain of the plastidic acetyl-CoA carboxylase gene are linked with resistance to herbicides in a *Lolium rigidum* population. *New Phytologist* **172**, 636–645.