



# Towards large-scale prediction of *Lolium rigidum* emergence. I. Can climate be used to predict dormancy parameters?

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## Summary

*Lolium rigidum* (annual or rigid ryegrass) is a widespread annual weed in cropping systems of southern Australia. Seeds exhibit dormancy at dispersal and require a period of dry after-ripening to release dormancy, before germination and emergence can occur. Climate, particularly temperature and rainfall, modulates dormancy level at seed maturity and dormancy release during after-ripening. This study investigated the possibility to predict seed dormancy of *L. rigidum* over a large scale, based on spatial and climatic factors. Mature seeds were collected from 406 populations across 15 different agronomic zones of 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. Logistic growth curve models were then fitted for each population, with the resulting equation used to estimate four further parameters describing dormancy status of the population. These parameters were used to determine relationships between location in the grain belt and long-term and current-year temperature and rainfall parameters for each population. Although some trends in seed dormancy patterns were found and distinct spatial clusters were clearly evident, our results indicate that climatic parameters alone are unlikely to be a useful predictor for seed dormancy in *L. rigidum* on a large scale, such as the Western Australian grain belt.

**Keywords:** annual ryegrass, survey, weed management, seed dormancy, germination, populations, weather.

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## Introduction

*Lolium rigidum* Gaud. (annual or rigid ryegrass) is a damaging weed in world agriculture and is the most economically significant weed in southern Australian cropping systems (cereal, oilseed rape and pulse/lupin crops) which are sown in early May and harvested late October/November. In the past, *L. rigidum* was grown

across the Australian grain belt as a pasture plant; however, it is now widely naturalised and often present at high densities. *Lolium rigidum* is a highly genetically variable cross-pollinated species and herbicide over-reliance has resulted in the widespread occurrence of herbicide-resistant populations. Increasing herbicide resistance levels (Owen *et al.*, 2007) and changes in farming systems, such as the adoption of minimum

tillage (D'Emden *et al.*, 2006), has enabled *L. rigidum* to persist across large areas and in a range of environmental and climatic conditions.

Seed dormancy can help to facilitate the persistence of weed species in farming systems by varying the timing of seed germination and seedling emergence (Forcella *et al.*, 2000). The majority of *L. rigidum* seeds are dormant when dispersed at the conclusion of the winter cropping period. Before germination and emergence can occur in the subsequent cropping season (May–October), a period of dry after-ripening is required to release dormancy (Steadman *et al.*, 2003a). The rate at which *L. rigidum* seeds lose dormancy during after-ripening is controlled mainly by temperature, being faster at higher temperatures (Steadman *et al.*, 2004b). Dormancy enables seeds to avoid germination during infrequent summer rainfall events. Seedlings would perish under the conditions associated with Mediterranean-type summers of southern Australia, which are characterised by high summer temperatures and drought.

Models that aim to study the economics of managing *L. rigidum*, such as Ryegrass Integrated Management (Pannell *et al.*, 2004), or that predict the development of herbicide resistance in *L. rigidum* (Neve *et al.*, 2003a,b) currently assume a single value for seed dormancy across populations. A value of 80% germination at the beginning of the winter cropping period is commonly used in these models. However, the release of seed dormancy and subsequent germination in *L. rigidum* populations is highly variable, with populations collected in different years or from different locations exhibiting vast differences in initial dormancy and the rate of dormancy release (Steadman *et al.*, 2003a,b). If model assumptions are based on limited data, then the projected outcomes may be significantly different to reality. Consequently, to improve the accuracy of data used in modelling approaches to integrated weed management, better understanding of seed dormancy across large cropping regions is necessary (Grundy, 2003).

Over many generations, natural selection in *L. rigidum* would favour seed dormancy characteristics that are optimal for plant survival. It has been determined that climate plays a pivotal role in modulating seed dormancy in *L. rigidum* (Steadman *et al.*, 2003a,b, 2004a,b), shown by experimental manipulation of the environment experienced by the parent plant during seed maturation (particularly temperature) and by the seeds during after-ripening, altering the dormancy characteristics of seeds (Steadman *et al.*, 2004b). Seeds from plants grown at warm temperatures were fewer in number, weighed less and were less dormant than those from plants grown at cool temperatures. Also, seeds that developed under cool temperature and reduced moisture conditions lost dormancy faster than seeds from

well-hydrated plants. Furthermore, a pilot study comparing seed dormancy characteristics of populations collected from 12 sites across the Western Australian grain belt indicated that certain long-term climate parameters and conditions experienced during seed development have the potential for use in dormancy prediction (Steadman *et al.*, 2004b). For example, higher September temperatures in the year of seed development and greater early long-term rainfall patterns, showed strong positive correlations with low seed dormancy, particularly the proportion of seeds remaining dormant following 5 months of after-ripening. Additionally, fewer seeds appeared to remain dormant when they were produced in areas that tend to have a greater proportion of rainfall prior to the growing season. Conversely, Chauhan *et al.* (2006) found that initial dormancy from five populations of *L. rigidum*, collected from different paddocks within a single farm in South Australia, had no variation in the level of seed dormancy ( $P = 0.37$ ), germination and viability, although the species is known for its very high genetic diversity.

To thoroughly investigate the potential to predict *L. rigidum* seed dormancy on a large scale, mature seeds were collected from 406 field populations distributed across the grain belt of southern Western Australia (WA), and the initial dormancy and change in dormancy over a 6-month period were measured. Location within the grain belt, long-term climatic parameters and the climate parameters during the year of seed development were compared with dormancy characteristics.

## 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 (Table 1). In total, 500 cropping fields were visited across 15 agronomic zones in the WA grain belt (Fig. 1), encompassing a 14 million hectare crop production region. Agronomic zones of the grain belt are divided according to annual average rainfall; H (High – 450–750 mm), M (Medium – 325–450 mm) and L (Low – less than 325 mm) and temperature zones; 1 (North), 2 (North central), 3 (Central), 4 (South central) and 5 (South). Crop fields were randomly visited at 5 km intervals (geo-referenced) in each zone, with mature *L. rigidum* seeds collected from each field. For more detailed sampling methods, see Owen *et al.* (2007). All samples were stored in paper bags in a ventilated glasshouse for 2–3 weeks, before being moved 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.

**Table 1** Sample number by agronomic zone and dates of collection and commencement of experiments. All populations were collected during November and December 2003. Seeds were stored in a glasshouse for 2–3 weeks and then a laboratory (23°C) for 1–2 weeks for processing before the initial germination test could be completed; temperature was logged and thermal time accumulation calculated. A base temperature of 5°C was used, with thermal time accumulation beginning from when the seeds first entered the glasshouse (1–2 days after seed collection)

Zone and number of populations	Date of seed collection	Date of first germination test	Thermal time at first germination test (°C)
L1 (21)	6th November	9th December	570
M1 (25) + H1 (29)	10–13th November	9th December	570
H2 (20)	25th November	16th December	444
M2 (28) + L2 (27)	19–21st November	16th December	444
H3 (25)	25–28th November	6th January	780
M3 (32) + L3 (24)	19–21st November	6th January	780
H4 (33)	3rd December	20th January	1028
M4 (30) + L4 (26)	24–27th November	20th January	1028
H5 (43) + M5 (30) + L5 (13)	1–6th December	22nd January	1069



**Fig. 1** Map of the south-west of WA indicating where the 406 populations of *Lolium rigidum* seeds were collected during November and December 2003. Agronomic zones of the grain belt are divided according to annual average rainfall; H (High – 450–750 mm), M (Medium – 325–450 mm) and L (Low – less than 325 mm) and temperature zones; 1 (North), 2 (North central), 3 (Central), 4 (South central) and 5 (South).

Of the 452 populations collected, 406 populations had sufficient seed numbers (more than 2 g of clean seed) to assess dormancy status and dormancy release.

#### After-ripening and germination testing

Freshly harvested seeds were placed in paper bags and stored in a ventilated glasshouse for after-ripening; air temperature was logged using a Tiny Tag Transit data logger (Gemini, Chichester, UK) located with the bags. Change in dormancy during after-ripening was assessed at monthly intervals, by removing 50 seeds from each of the 406 populations for germination testing. Seeds were

germinated in 9 cm diameter Petri dishes containing 1% (w/v) solidified agar-water; one Petri dish containing 50 seeds was germinated monthly for each population. Petri dishes were placed in a controlled environment growth cabinet set at 25/15°C (light/dark, 12 h daily photoperiod of 30–60  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , cool white fluorescent light), optimal for germination (Steadman, 2004). The number of germinated seeds were counted after 28 days, with the criterion for germination being visible radicle protrusion. Non-germinated seeds were assessed for viability by gently pinching, to determine if they contained a living, turgid embryo [pilot studies by Goggin *et al.* (2008) have demonstrated that this test gives the same results as tetrazolium staining] and non-viable or empty seeds were deducted from the total number of seeds used in the test. Viability of all populations (tested by visual inspection, and empty seeds excluded at the time of set-up) was greater than 95%.

#### Statistical analysis

All statistical analysis was carried out in the R programming language (R Development Core Team, 2007). Logistic regression was used to model release of dormancy as a function of after-ripening. The temperature experienced during after-ripening was variable (Table 1) as seeds were germinated at different times due to the large number of populations, therefore the time scale was converted to thermal time (Steadman *et al.*, 2003b). A base temperature for dormancy release of 5°C was used based on previous research which suggests a value in this region for both dormancy and germination processes in *L. rigidum* (Steadman *et al.*, 2003a,b). Thermal time was expressed as cumulative day degrees from the time seeds were placed in the glasshouse (approximately 1–3 days after seed collection, when

seeds had reached maturity; a time that was similar for all populations).

Separate three-parameter logistic growth curve models were fitted for each population. This led to a total of 406 logistic models of the form:

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

where  $G_i$  is the percentage of seeds germinating at thermal time  $t_i$  and  $t_i - t_0$  is the number of day degrees of after-ripening since the start of the experiment ( $t_0$ ) experienced by the sample taken at  $t_i$  day degrees. The parameter  $G_{\max}$  represents the upper bound on the percentage of seeds losing dormancy as thermal time becomes large (the asymptote) and is constrained to be less than or equal to 100, the slope parameter dormancy release rate (DRR) describes the DRR, and  $b$  is a 'horizontal shift' parameter closely related to the germination percent at  $t_0$  (Steadman *et al.*, 2004b). The three parameters  $G_{\max}$ , DRR and  $b$  were thus estimated separately for each of the 406 populations, using the R function *nlm* to minimise negative log-likelihood. 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:  $G_{1069}$ ,  $G_{4000}$  and  $G_{\text{change}}$ . The first of these parameters,  $G_{1069}$ , is the estimated germination 1069 day degrees after seed collection and the time when all populations had been tested, and compares all populations at the same point in time (Table 1).  $G_{1069}$  was chosen as a measure of population germinability in summer, at the beginning of after-ripening, and hereafter is called 'summer germinability'. The second parameter,  $G_{4000}$ , is the estimated germination 4000 day degrees after seed collection and the latest time at which all populations were still to have a final germination test.  $G_{4000}$  was chosen as a measure of population germinability in winter, after a substantial period of after-ripening, and is called hereafter 'winter germinability'. We chose to use  $G_{4000}$  rather than  $G_{\max}$  as a measure of winter germinability, because initial data exploration showed that  $G_{\max}$  was

extremely sensitive to data outliers. A third parameter,  $G_{\text{change}}$ , was recorded as a measure of the relative change in germinability from summer to winter, where  $G_{\text{change}}$  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 day degrees, but had lost dormancy by 4000 day degrees. To represent the dormancy characteristics of each population, we chose to use the three calculated parameters,  $G_{1069}$ ,  $G_{4000}$  and  $G_{\text{change}}$ , in addition to DRR as a fourth parameter that describes the speed of release of seed dormancy for each population. We also investigated the relationship between the selected four dormancy parameters described above by determining their correlation with each other.

An extensive analysis was then conducted to look for relationships between the four dormancy status parameters for each population and (i) spatial patterns on a map, (ii) latitude and longitude, (iii) rainfall zone (H/M/L), temperature zone (1/2/3/4/5), agronomic zone and (iv) climate data. The climate data used for comparison step (iv) were calculated from long-term records (30 year averages) of the daily rainfall and maximum and minimum temperatures recorded by automatic weather stations (Australian Bureau of Meteorology, 2003). Climate data were obtained from a weather station as close as possible to the seed collection site. Seventeen parameters were calculated from the data, either specific to the season in which maternal plant growth and seed development occurred, or long-term averages based on historical (30 years) data (Table 2).

When considering the ability of the covariates to predict each of the following dormancy parameters ( $G_{1069}$ ,  $G_{4000}$  and  $G_{\text{change}}$ ), we fitted a generalised linear model (GLM) with a logistic link function and quasi-binomial errors. Since the predicted variable in this case

**Table 2** Climate parameters calculated from long-term (30-year averages) records of daily rainfall and maximum and minimum temperatures collected by weather stations across southern WA

During year of seed development	Long-term climate
(1) Average September temperature	(11) Average January–March (summer) temperature
(2) Average October temperature	(12) Total January–March rainfall
(3) Average November temperature	(13) Proportion of early to total growing season rainfall (April–June/April–October)
(4) Average September–November temperature	(14) Average April–October (growing season) temperature
(5) Average April–October (growing season) temperature	(15) Total April–October rainfall
(6) Total September rainfall	(16) Average September–November temperature
(7) Total October rainfall	(17) Total September–November rainfall
(8) Total November rainfall	
(9) Total September–November rainfall	
(10) Total April–October rainfall	

was binomial (germination proportion) it was most appropriate to use a model with binomial errors. The quasi-binomial model was used in preference to a straight binomial GLM, because significant over-dispersion was found in the data. When considering the ability of the covariates to predict the DRR, a negative log-transformation was made (which normalised errors) and then a linear model was fitted against the transformed variable  $-\log(-\text{DRR})$ . Data presented is 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. All tests were conducted at the  $P = 0.05$  significance level unless stated otherwise.

## Results

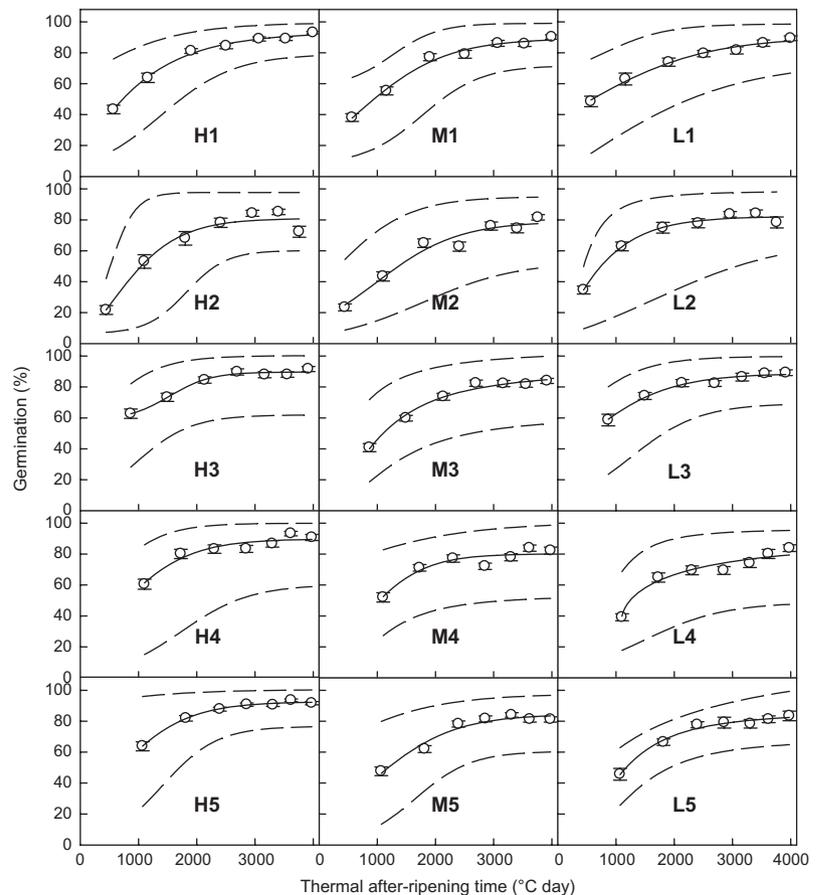
For seeds of all 406 ryegrass populations collected at maturity from crop fields across the WA grain belt, increasing proportions of seeds were able to germinate as after-ripening progressed. However, the change in

seed germinability with increasing thermal after-ripening time was highly variable among populations (Fig. 2). Four dormancy parameters were calculated for each population; summer germinability ( $G_{1069}$ ), winter germinability ( $G_{4000}$ ), relative change in germinability ( $G_{\text{change}}$ ) and DRR.

### Relationship between dormancy parameters

Summer germinability ( $G_{1069}$ ) status of a population was a significant predictor of the winter germinability ( $G_{4000}$ ) of a population, although populations that had low germination in summer did not necessarily have low germination in winter. Each dormancy parameter was significantly positively correlated ( $P < 0.01$ ) with each of the other three parameters (Table 3).

DRR and relative change in germinability ( $G_{\text{change}}$ ) were related significantly; however, their correlation was relatively low. Whereas DRR measures the speed in release of dormancy (i.e. fast or slow) from  $t_0$  to  $G_{\text{max}}$  (Fig. 2),  $G_{\text{change}}$  measures the relative change in germinability from summer ( $G_{1069}$ ), when the majority of populations had lost partial dormancy, to winter ( $G_{4000}$ ). For example, H1 populations increased in germinability from an average of 64% in summer ( $G_{1069}$ ) to 93% in



**Fig. 2** Change in germination (% of viable seeds) with increasing thermal after-ripening time for 406 *Lolium rigidum* populations collected from the grain belt of WA during November and December 2003. Seeds were after-ripened in a glasshouse and retrieved each month over a 6-month period and germinated at 25/15°C (12 h daily photoperiod). Populations were grouped according to agronomic zone and logistic regressions plotted for average germination (solid line), with the populations with the highest values and lowest values also shown (dotted lines). Bars are  $\pm$  SE of the mean. Thermal after-ripening time corresponds to the collection date (see Table 1 for details).  $P$ -values are presented in Tables 4 and 5.

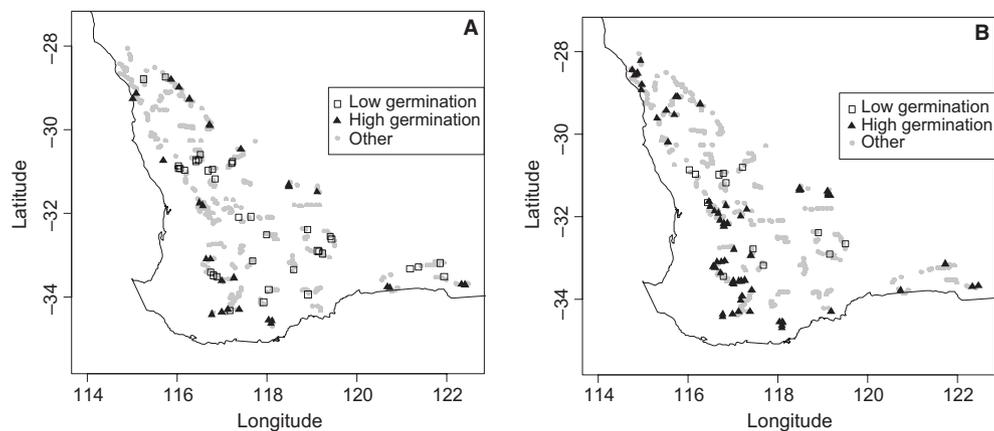
**Table 3** Relationship and correlations between the dormancy parameters ( $G_{1069}$ , summer germination;  $G_{4000}$ , winter germination;  $G_{\text{change}}$ , change in germination between summer and winter; DRR, dormancy release rate). Values are unadjusted correlations between the pairs of germination parameters, with all values significantly ( $P < 0.01$ ) linearly related when fitting a model between the pairs of dormancy parameters

	$G_{1069}$	$G_{4000}$	$-\log(-\text{DRR})$
$G_{4000}$	0.72		
$-\log(-\text{DRR})$	0.15	0.23	
$G_{\text{change}}$	0.15	0.72	0.22

winter ( $G_{4000}$ ), so  $G_{\text{change}}$  would be relatively small, even though DRR might be fast. This also explains why summer germinability ( $G_{1069}$ ) had a weak correlation with either DRR or relative change in germinability ( $G_{\text{change}}$ ), as most populations had already experienced partial dormancy loss at  $G_{1069}$ . Winter germinability ( $G_{4000}$ ) was correlated significantly with all other parameters and is the most useful parameter for predicting dormancy characteristics in the population. It also has the advantage of representing the proportion of seeds that would germinate at the start of the growing season in WA.

#### Latitude and longitude

Spatial clusters were clearly visible when comparing summer ( $G_{1069}$ ) (Fig. 3A) and winter germinability ( $G_{4000}$ ) (Fig. 3B). For example, populations with low summer germinability occurred within Zone M2 (Fig. 3A), high winter germinability populations were found in Zone H4 (Fig. 3B), and a cluster of populations with large changes in germination were present in the far south-west (data not shown). Similar spatial patterns



**Fig. 3** Maps showing the locations of populations with low and high summer (A) and winter (B) germinability. Summer germinability is based on the  $G_{1069}$  parameter for each population, with high germinability defined as  $> 80\%$  germination and low germinability defined as  $< 30\%$  germination. Winter germinability is based on the  $G_{4000}$  parameter for each population, with high germinability defined as  $> 95\%$  germination and low germinability as  $< 65\%$  germination.

were observed for the other parameters, DRR and  $G_{\text{change}}$  (data not shown). Whilst clusters were apparent, there were no exclusive patterns and they remained scattered throughout the zones. Additionally, there was an absence of any major relationship between dormancy status parameters and spatial coordinates (latitude/longitude) (Table 4).

#### Zone

Temperature, rainfall or agronomic zone (see Fig. 1) had a significant effect on most dormancy parameters (Table 5). When these effects were investigated in more depth, there were differences in germination between the zones in relation to temperature and rainfall, but no consistent trends could be determined (Fig. 4). In fact, the differences in dormancy characteristics could be tracked to the level of the agronomic zones from which the ryegrass populations were collected. Populations from Zones L4, L5, M5 and M2 had lower summer germination than other zones ( $G_{1069} = 42\text{--}48\%$ ), while L3, H1, H3 and H5 had the highest summer germination ( $G_{1069} = 62\text{--}72\%$ ) (Fig. 5A). Winter germinability was similar, with the populations from Zones H1, H3, H4 and H5 having high levels of germination, while those from Zone M2 germinated the least (Fig. 5B).

#### Climate

There was a significant relationship observed between summer germinability ( $G_{1069}$ ) and September temperature in the year of seed production (Table 4), with increasing temperatures associated with greater summer germination. Winter germination ( $G_{4000}$ ) was not correlated with any temperature parameter. Long-term total

**Table 4** Significance of relationships between continuous variables (spatial co-ordinates, climate) and dormancy status parameters ( $G_{1069}$ , summer germination;  $G_{4000}$ , winter germination;  $G_{\text{change}}$ , change in germination between summer and winter; DRR, dormancy release rate) as modelled by a quasibinomial GLM with logistic link function. For significant results ( $P < 0.05$ ),  $P$ -values are given and + or – sign indicates the direction of the relationship, while n.s. indicates  $P > 0.05$  (not significant)

Continuous variable	$G_{1069}$	$G_{4000}$	$G_{\text{change}}$	–log(–DRR)
<i>Spatial co-ordinates</i>				
Latitude	n.s.	n.s.	0.009–	n.s.
Longitude	n.s.	n.s.	n.s.	n.s.
<i>2003 average temperature (°C)</i>				
September	0.044 +	n.s.	0.008–	n.s.
October	n.s.	n.s.	0.001–	n.s.
November	n.s.	n.s.	0.001–	n.s.
September–November	n.s.	n.s.	0.001–	n.s.
April–October (growing season)	n.s.	n.s.	0.012–	n.s.
<i>2003 total rainfall (mm)</i>				
September	n.s.	n.s.	0.004+	n.s.
October	n.s.	n.s.	0.02+	n.s.
November	n.s.	n.s.	n.s.	n.s.
September–November	n.s.	n.s.	0.024+	n.s.
April–October (growing season)	n.s.	n.s.	0.016+	n.s.
<i>Long-term average temperature (°C)</i>				
January–March (summer)	n.s.	n.s.	0.001–	n.s.
April–October (growing season)	n.s.	n.s.	0.007–	n.s.
September–November	n.s.	n.s.	<0.001–	n.s.
<i>Long-term total rainfall (mm)</i>				
January–March (summer)	n.s.	0.008–	0.005–	n.s.
Proportion of early to growing season rainfall (April–June/April–October)	n.s.	0.004–	<0.001–	n.s.
April–October (growing season)	n.s.	0.046+	n.s.	n.s.
September–November	n.s.	n.s.	<0.007+	n.s.

**Table 5** Significance of relationships between discrete variables (temperature zone, rainfall zone, agronomic zone) and dormancy status parameters ( $G_{1069}$ , summer germination;  $G_{4000}$ , winter germination;  $G_{\text{change}}$ , change in germination between summer and winter; DRR, dormancy release rate) as modelled by a quasibinomial GLM with logistic link function. For significant results ( $P < 0.05$ ),  $P$ -values are given, while n.s. indicates  $P > 0.05$  (not significant)

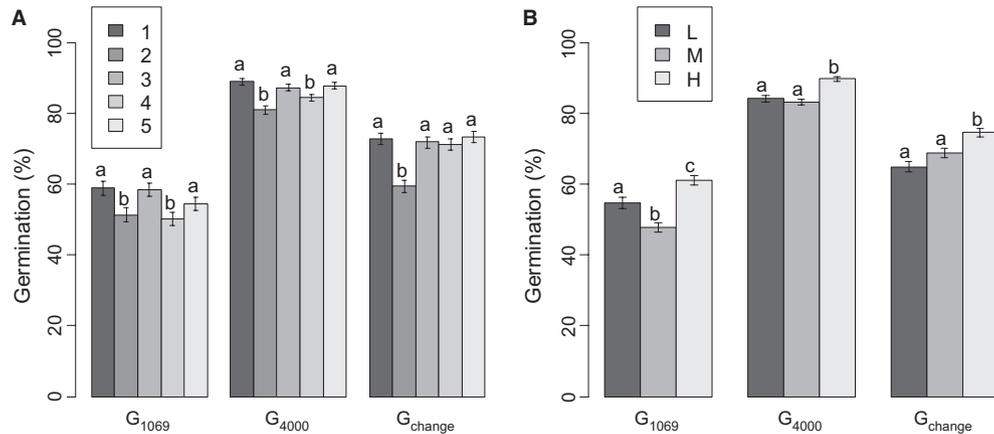
Discrete variable	$G_{1069}$	$G_{4000}$	$G_{\text{change}}$	–log(–DRR)
<i>Zone</i>				
Temperature	0.002	<0.001	<0.001	0.006
Rainfall	<0.001	<0.001	<0.001	n.s.
Agronomic	<0.001	<0.001	<0.001	0.008

rainfall influenced the winter dormancy ( $G_{4000}$ ) status of *L. rigidum* populations (Table 4). However, rainfall during the year of seed development did not appear to have any effect. Greater long-term rainfall during the growing season resulted in higher winter germination ( $G_{4000}$ ), whilst a higher proportion falling earlier in the growing season was associated with lower winter germination. Increased long-term summer rainfall was associated with populations having lower winter germination ( $G_{4000}$ ). A variety of significant interactions were observed for climate parameters with  $G_{\text{change}}$ , for example higher temperatures being associated with smaller  $G_{\text{change}}$  and higher rainfall being associated with larger  $G_{\text{change}}$ .

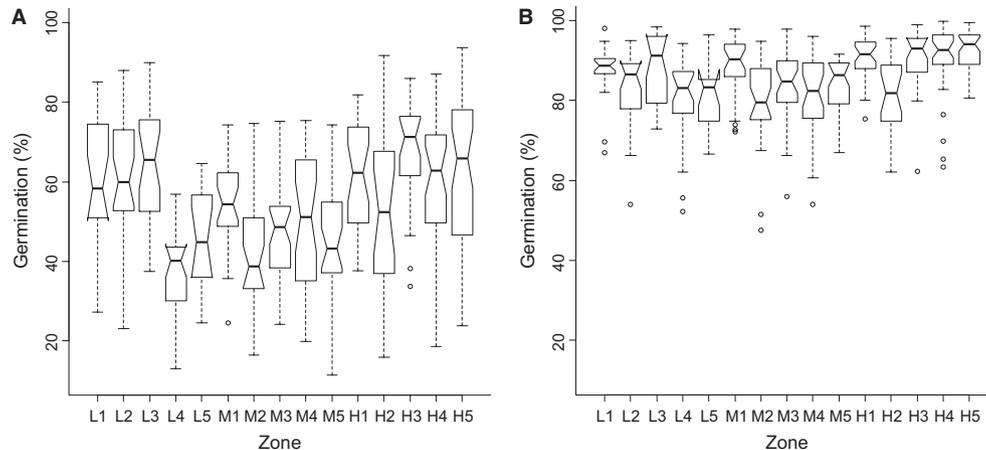
## Discussion

The highly variable nature of seed dormancy in *L. rigidum* (Steadman *et al.*, 2003a,b) was again confirmed in the present study of 406 populations collected from across the 14 million hectare grain belt of WA. Although strong indications for a link with climate (Steadman *et al.*, 2003a,b, 2004a,b) suggested the possibility to predict seed dormancy status between growing seasons which could improve management of weed seedbanks (Steadman *et al.*, 2004b), this study has shown that when given an extensive area with a large number of *L. rigidum* populations, it is difficult to find consistent relationships with climate that could be used to predict the seed dormancy release pattern over an entire area or agricultural region. This is partially because it was fundamentally difficult to compare germination at seed maturity or even to formulate a single comparable measure of ‘initial germinability’, as populations matured across a wide range of geographical locations and at different times (i.e. between north and south, east and west). This is further exacerbated by the time spent collecting populations across the entire grain belt prior to crop harvest at each location, and transporting seeds back to the experimental site.

Temperature during seed development (September) was a strong indicator of summer dormancy ( $G_{1069}$ ), with lower temperatures linked to higher dormancy levels. The maternal environment plays a pivotal role in determining seed dormancy at maturity (particularly in grass species), with seeds developing in low temperatures generally more dormant than those in hotter temperatures (Boyce *et al.*, 1976; Fenner, 1991; Baskin & Baskin, 1998). Within *Lolium* species, seeds from plants grown under cooler temperatures were more dormant when measured at harvest and following five months of after-ripening (Wiesner & Grabe, 1972; Steadman *et al.*, 2004b). This is consistent with the



**Fig. 4** Plots showing dormancy parameters for each (A) temperature zone and (B) rainfall zone. Temperature zones are split according to latitude and temperature (1–5) and rainfall zones are indicated by Low, Medium and High categories (see Fig. 1). The height of the shaded bar represents the average percentage germination as estimated by the fitted GLM, and the error bar represents the standard error for this estimate. Letters (a, b, c) indicate which values are significantly different ( $P < 0.05$ ) according to the fitted GLM.



**Fig. 5** Plots showing (A) summer ( $G_{1069}$ ) and (B) winter ( $G_{4000}$ ) germinability for each agronomic zone (see Fig. 1). Bars indicate median values, boxes indicate inter-quartile range, dotted bars (whiskers) indicate 1.5 times the inter-quartile range. Data points beyond this range are shown separately as outliers. Notches indicate approximate 95% confidence intervals; when notches do not overlap there is strong evidence that the two medians truly differ (Chambers *et al.*, 1983; R Development Core Team, 2007).

findings of our study, although there was no significant relationship between temperature and dormancy level in winter following after-ripening.

Winter germinability ( $G_{4000}$ ) was highly correlated to all other dormancy parameters and is the most useful and relevant predictor of dormancy in this study. It has the advantage of indicating the potential seedling emergence of each population at a time that is optimal for plant growth and development in Mediterranean-type climates such as WA, when water is non-limiting and temperature conditions are most favourable (Bell *et al.*, 1993). Winter germinability exhibited no relationship with growing season temperature, long-term temperature, latitude or longitude. However, long-term rainfall conditions were a strong predictor of winter

dormancy, with winter dormancy levels greatest in plants from areas with high summer rainfall and a high proportion of rain falling in the early growing season. These conditions represent precarious environments for seedling establishment because, in summer, rainfall can be inconsistent, and in the early growing season, many plants compete for limited resources. Thus, *L. rigidum* has adapted to survive these risky conditions by limiting the proportion of seeds capable of germination during any one growing season. This contradicts a small-scale pilot study of 12 populations, where winter germinability was higher in areas that have a higher proportion of rainfall falling early in the year (Steadman *et al.*, 2004b). This was attributed to summer or early season rainfall reducing the need for *L. rigidum* to conserve seed for

future years, through the replenishing of soil moisture prior to the growing season. However, long-term growing season rainfall was also a strong predictor of winter dormancy, with seed populations from areas with higher growing season rainfall having higher germination in winter. Therefore, our study has shown that *L. rigidum* populations have adapted to long-term climatic conditions by producing more dormant seeds under 'riskier' conditions allowing seed survival for future seasons. For example, higher levels of seed dormancy at maturity have been associated with environments that have a higher probability of summer storms, which could trigger premature germination into an environment that may be lethal (Meyer *et al.*, 1997).

There were distinct spatial clusters of populations clearly evident in the south-western agricultural region. For example, the populations that retained the greatest dormancy through to winter were located in the eastern region, with Zone M2 having populations with the highest dormancy. However, a population found in Zone M3, also in a similar region, was associated with very low winter dormancy. We conclude that while climate has an important role in modulating dormancy at the individual plant level, climatic parameters alone are unlikely to be useful for predicting seed dormancy in *L. rigidum* at the large scale of the Western Australian grain belt. Previous work has shown that local environmental conditions may in fact override the effects of climate (Petru & Tielborger, 2008), with variable climatic conditions experienced in individual fields, each with its own unique selection pressure, likely to influence dormancy patterns. Crop management factors such as tillage and rotations can also affect seed dormancy by influencing the micro-climate conditions surrounding seeds in soil (Dyer, 1995). For example, the environmental conditions that the mother plants are exposed to, such as precipitation, temperature, altitude, day length, and soil moisture, can influence dormancy levels (Andersson & Milberg, 1998 and references therein; Baskin & Baskin, 1998). Variations in the micro-climate such as nutrient supply, soil characteristics including interactions with soil hydric conditions, water and light availability during seed development may govern changes in the degree of dormancy (Benech-Arnold *et al.*, 2000).

The highly variable nature of seed dormancy in our study may be a reflection of the variability in the large number of populations collected across a 14 million hectare region. Although the conditions for dormancy loss are species specific, variability in dormancy behaviour exists even within a species (Grundy, 2003), with *L. rigidum* being well known for its high genetic diversity. A previous study by Steadman *et al.* (2004b), which suggested the potential for a predictive model of *L. rigidum* germination based on consistent relationships

between seed dormancy and climatic conditions, only considered 12 populations in the WA grain belt. The current study considered more than 400 populations, with a large number of populations from a single climatic zone. This resulted in large variations that cannot be explained by climate alone, as each field is subject to different evolutionary events affected by climate, management practices and genetic background. The existence of a wide range of interactions among factors that affect dormancy in the field, including environmental and agricultural variables, indicate that the development of models predicting dormancy status is an extremely difficult task [as reviewed in Benech-Arnold *et al.* (2000)].

In summary, while it is difficult to predict the dormancy status of a particular population or plant, there are strong trends, suggesting some degree of generalisation is possible. While climate may play an important role in determining dormancy status, our results indicate that the use of climatic parameters as a predictive tool for seed dormancy in *L. rigidum* at the large-scale grain belt level is inconsistent. However, other factors, such as the herbicide resistance status of populations, may play a potential role in the prediction of seed dormancy in *L. rigidum*. The implications of this are explored in the accompanying paper (Owen *et al.*, in press), which examines the link between seed dormancy status and herbicide resistance status.

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