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Incidence of Herbicide Resistance, Seedling Emergence, and Seed Persistence of Smooth Barley (*Hordeum glaucum*) in South Australia

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Smooth barley has emerged as a problematic weed in cereal crops of South Australia. After the recent reports of herbicide resistance and increase in seed dormancy in smooth barley, it was considered important to determine the herbicide resistance status and seedbank behavior of field populations of this weed species. A field survey was undertaken in the Upper North and Eyre Peninsula regions of South Australia in October 2012. Of the 90 smooth barley populations screened for resistance to quizalofop, 15% exhibited some level of resistance and 85% were susceptible. Resistance to acetolactate synthase (ALS)-inhibiting herbicides was low, with only 3 and 12% of populations classified as developing resistance to imazamox + imazapyr and sulfosulfuron, respectively. No multiple resistance patterns were observed; however, two ALS-inhibiting herbicide-resistant populations had sulfonylurea-to-imidazolinone cross-resistance. At the start of the growing season, the majority of smooth barley populations emerged rapidly (median 50% time to emergence [T_{50}] = 8 d). In contrast, some populations of smooth barley displayed an extremely slow emergence pattern, with T_{50} of > 20 d. No direct linkage between seed dormancy and herbicide resistance was observed. However, two acetyl coenzyme A carboxylase-inhibiting herbicide-resistant populations were highly dormant and exhibited delayed emergence. The majority of smooth barley populations showed low-level or no seedbank persistence, but a few populations persisted for 1 yr. However, some weed populations had up to 20% seedbank persistence from 1 yr to the next. Overall there was a strong negative relationship between smooth barley seedling emergence and the level of seed persistence (R^2 = 0.84, $P < 0.05$). This association indicated that greater seed dormancy could be responsible for extended persistence of the seedbank of this weed species. The study provides valuable insights into the general pattern of herbicide resistance and the behavior of the seedbank of smooth barley populations on South Australian farms.

Nomenclature: Imazamox + imazapyr; quizalofop; sulfosulfuron; smooth barley, *Hordeum glaucum* (Steud.) Tzvelev.

Key words: ACCase-inhibiting herbicide, ALS-inhibiting herbicide, herbicide resistance, seed dormancy, seedbank persistence, seedling emergence.

Hordeum glaucum ha emergido como una maleza problemática en los cultivos de cereales en el Sur de Australia. Después de reportes recientes de resistencia a herbicidas y el incremento en la dormancia de la semilla en *H. glaucum*, se consideró importante determinar el estatus de la resistencia a herbicidas y el comportamiento del banco de semillas de poblaciones de campo de esta especie. Se realizó un estudio observacional de campo en las regiones Alta Norte y de la península Eyre en el Sur de Australia, en Octubre 2012. De las 90 poblaciones de *H. glaucum* evaluadas por resistencia a quizalofop, 14% exhibieron algún nivel de resistencia y 86% fueron susceptibles. La resistencia a herbicidas inhibidores de acetolactate synthase (ALS) fue baja, ya que solamente 3 y 12% de las poblaciones fueron clasificadas como desarrollando resistencia a imazamox + imazapyr y sulfosulfuron, respectivamente. No se observó ningún patrón de resistencia múltiple. Sin embargo, dos poblaciones resistentes a herbicidas inhibidores de ALS tuvieron resistencia cruzada de sulfonylurea a imidazolinone. Al inicio de la temporada de crecimiento, la mayoría de las poblaciones de *H. glaucum* emergieron rápidamente (mediana del tiempo de 50% de emergencia [T_{50}] = 8 d). En contraste, algunas poblaciones de *H. glaucum* mostraron un patrón de emergencia extremadamente tardío, con T_{50} de > 20 d. No se observó ninguna relación directa entre la dormancia de la semilla y la resistencia a herbicidas. Sin embargo, dos poblaciones resistentes a herbicidas inhibidores de acetyl coenzyme A carboxylase tuvieron una alta dormancia y exhibieron un retraso en la emergencia. La mayoría de las poblaciones de *H. glaucum* mostraron de bajo a ninguna persistencia del banco de semillas, pero algunas poblaciones persistieron por 1 año. Sin embargo, algunas poblaciones tuvieron hasta 20% de persistencia del banco de semillas de un año al otro. En general, hubo una fuerte relación negativa entre la emergencia de plántulas de *H. glaucum* y el nivel de persistencia de la semillas (R^2

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= 0.84, P < 0.05). Esta asociación indicó que una mayor dormancia de la semilla podría ser responsable por la persistencia extendida del banco de semillas de esta especie de maleza. Este estudio brinda una observación valiosa sobre el patrón general de resistencia a herbicida y el comportamiento del banco de semillas de poblaciones de *H. glaucum* en fincas del Sur de Australia.

Herbicide resistance and seed dormancy in weeds are striking examples of evolution in agricultural systems. Intense selection pressure imposed on genetically diverse weed populations provides stimuli for rapid evolution of herbicide resistance (Maxwell and Mortimer 1994). If traits such as herbicide resistance are present in a genetically variable natural population, even at low frequencies, the recurrent selection of these traits with the repeated herbicide application will increase the frequency of resistant individuals (Jasieniuk and Maxwell 1994; Owen et al. 2014). The same analogy can be used to explain the selection of highly dormant individuals in a cropping system in which early-emerging individuals are effectively killed by tillage or non-selective herbicides.

Herbicide-resistant weeds are a major problem in the cropping regions of Australia. Currently, there are over 75 weed species that have evolved herbicide resistance in Australia (Heap 2015). Widespread adoption of minimum tillage, because of its benefits such as reduced soil erosion, reduced fuel and labor cost, and timely sowing, and heavy reliance on herbicides has significantly contributed to the rapid appearance of herbicide resistance (Pratley 1995; Walsh and Powles 2007)

Smooth barley is an ubiquitous weed in the annual pasture zone of southern Australia (Cocks et al. 1976; Smith 1968). Previous studies have shown that this species has short-lived innate dormancy, and was unlikely to be problematic in crops as the majority of the seeds germinated with early autumn rains (Davison 1971; Harris 1961; Smith 1968) and the seedbank did not persist from one year to the next (Popay 1981). In a later study, Fleet and Gill (2010) showed that farming practices used in southern Australia have selected smooth barley populations that possess high levels of seed dormancy, which is broken by the exposure to cold temperatures in winter. This is an effective escape mechanism that allows some plants in these populations to avoid pre-sowing non-selective herbicides and establish after the crops have been planted. In the past, non-dormant populations of smooth barley could be easily controlled with the use of burndown herbicides applied in late autumn. But this change in weed biology has increased the selection pressure on POST herbicides, as these are being increasingly relied on for the control of smooth barley in crops. Furthermore, investigations of populations of smooth barley and a closely related species hare barley (*Hordeum leporinum* L.) have confirmed resistance to acetyl coenzyme A carboxylase (ACCase)-inhibiting herbicides in South Australia and Tasmania (Broster et al. 2012; Matthews et al. 2000; Shergill et al. 2015), to acetolactate synthase (ALS)-inhibiting herbicides in Western Australia (Owen et al. 2012; Yu et al. 2007), and to bipyridyliums across southern Australia (Hidayat 2004; Owen et al. 2012; Powles 1986; Preston et al. 1992). The combination of increased seed dormancy with herbicide resistance would make it very difficult for Australian farmers to effectively manage this weed species in cropping systems.

At this stage it is unclear whether there is any link between increased seed dormancy and increased herbicide resistance in smooth barley. A link between herbicide resistance and seed dormancy in other species has been reported occasionally (Ghersa et al. 1994; Gill et al. 1996; Owen et al. 2014; Recasens et al. 2007; Tranel and Dekker 2002; Vila-Aiub et al. 2005). Therefore, detailed knowledge of seed biology, particularly timing of seedling emergence and seedbank persistence, is required for the development of integrated weed management practices. It is also important to quantify the occurrence of herbicide resistance, which should also aid in the development of effective weed management systems.

In this paper we report the findings of a random survey of cropping fields across the grain-cropping regions of South Australia. The objectives of the studies reported here were to (1) quantify the occurrence of herbicide resistance in smooth barley populations across South Australia, (2) determine the level of variation in seed dormancy and persistence in smooth barley populations across

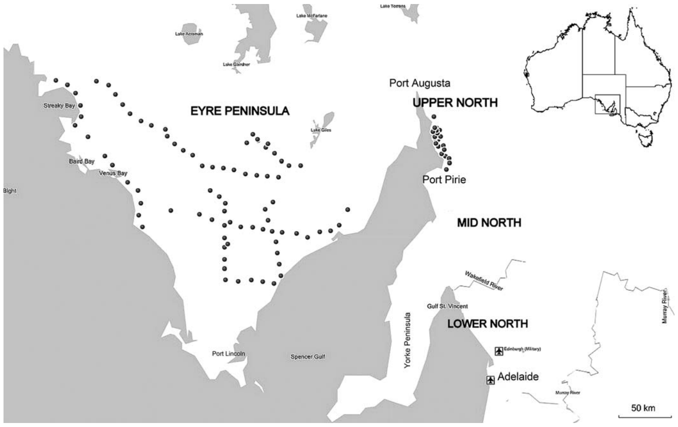

Figure 1. Map of surveyed agronomic regions (names in capital letters) across South Australia, showing the location (•) of surveyed fields where smooth barley populations were collected for herbicide resistance screening and weed biology studies.

South Australia, and (3) examine the relationship between seed dormancy and herbicide resistance in populations of smooth barley. This study also documents the first known case of resistance to ALS-inhibiting herbicides in Australian populations of smooth barley.

Materials and Methods

Collection of Plant Material. Smooth barley generally starts flowering in August–September and produces mature seed by October, well before grain harvest in November–December. Therefore, a 3-wk field survey before grain harvest was conducted in October 2012. The survey focused on cropping fields in the upper north (UN) and Eyre Peninsula (EP) regions of South Australia (Figure 1). Fields were selected randomly and without any prior knowledge of herbicide history by traveling a predetermined distance of 5 km (UN region) or 10 km (EP region) along major and minor roads and then surveying the nearest crop or pasture field. At each stop a single field was surveyed. Fields were surveyed by two people moving in different directions; each followed an inverted W pattern through at least 1 ha of the field, beginning at least 20 m from the edge of the crop. For fields with patchy distribution of smooth barley, a representative sample was obtained by collecting approximately equal amounts of seed from most of the patches in the field. Sampling was discontinued once three-quarters of a 20-L bucket was full of seeds or panicles or after 30 min, whichever occurred first. After collection, seeds or panicles obtained were bulked to obtain a single sample and designated as a single population. The sample thus obtained was placed in a labeled paper bag and global positioning system (GPS) coordinates were recorded from a handheld GPS unit (Garmin eTrex Vista®, Garmin Australasia, Eastern Creek, New South Wales). Additional information of the crop being cultivated and visual smooth barley severity/distribution were also recorded and a weed distribution score was given to each sampled field. Immediately after collection, the seed samples were stored in the laboratory under dry conditions at room temperature at The University of Adelaide, Roseworthy Campus (34.52°S, 138.68°E), until February 2013 when they were threshed and cleaned manually.

Herbicide Resistance Screening. Resistance screening was conducted twice for ACCase-inhibiting and ALS-inhibiting herbicides in the normal growing season from May to August 2013. Only the populations that survived in the first screening were included in the second screening. Seeds of each of the 90 populations were germinated in plastic trays (33 cm by 28 cm by 5 cm) containing standard University of California (UC) potting mix (pasteurized potting soil based on a 60 : 40 mix of sand and peat moss). Germinated seedlings (10 per pot with three replicates) were transplanted into 9.5-cm by 8.5-cm by 9.5-cm punnet pots (Masrac Plastics, South Australia) containing the potting mix. A standard susceptible smooth barley population, Yaninee (from EP, South Australia) (Shergill et al. 2014) and a previously confirmed ACCase-inhibiting herbicide-resistant smooth barley population, F.P (from Baroota, South Australia), were used as susceptible and resistant controls. These populations were screened with ACCase and ALS-inhibiting herbicides.

ACCase-Inhibiting Herbicides. A commercial formulation of the most commonly used aryloxyphenoxypropionate (APP) ACCase-inhibiting herbicide quizalofop (Targa®, 99.5 g L^{-1}, Sipcam Pacific Australia Pty Ltd., Geelong, Victoria) at 24.9 g ai ha^{-1} was sprayed using a moving-boom laboratory twin-nozzle cabinet sprayer (Tee-jet 110° flat-fan spraying systems, Wheaton, IL) delivering herbicide in 121 L ha^{-1} water at a pressure of 250 kPa and a speed of 1 m s^{-1}. All populations were sprayed with commercial formulation plus 0.2% v/v BS1000 adjuvant (Crop Care Australasia Pty Ltd., Queensland) at the Z12–Z13 stage (Zadoks et al. 1974). Plants were returned and maintained outdoors after herbicide application and assessed for survival at 28 d after treatment. The plants with new green leaf tissue were recorded as resistant, whereas plants that displayed severe chlorosis or no new growth were recorded as susceptible. The populations were classified as resistant if 20% or more of the individuals in the population survived herbicide application. The populations with 1 to 19% survival were classified as developing resistance. Where there was less than 1% survival, the populations were classified as susceptible.

ALS-Inhibiting Herbicides. Because of poor germination of some populations, 74 populations were screened with the ALS-inhibiting herbicides. The populations were screened with sulfonylurea herbicide sulfosulfuron (Monza®, 750 g kg^{-1}, Nufarm Australia Ltd., Victoria) at 18.7 g ai ha^{-1} and the imidazolinone herbicide mixture imazamox plus imazapyr (Intervix®, 33 g L^{-1} and 15 g L^{-1}, Crop Care Australasia) at 24.8 plus 11.3 g ai ha^{-1}. Herbicides were applied as commercial formulation plus adjuvant; Hasten™ adjuvant (Victorian Chemical Co. Pty. Ltd., Victoria) was added to sulfosulfuron spray solution at 1% v/v; and BS1000 adjuvant was added to imazamox plus imazapyr spray solution at 0.2% v/v. All herbicides were applied at Z11–Z12 stage (Zadoks et al. 1974) with the same laboratory herbicide sprayer described above. The plants were also assessed and classified as described above.

Seedling Recruitment. In the next autumn (April 2013) after the seed collection, seeds (1 g each with three replicates) of 63 smooth barley populations with Yaninee as the standard control (susceptible and non-dormant) were sown in plastic trays (33 cm by 28 cm by 5 cm) containing standard potting UC mix and were maintained outside during the normal growing season at The University of Adelaide, Roseworthy Campus. To estimate weed seedling establishment, emergence counts (at first leaf appearance) were recorded from April until the end of October (7 mo or 190 d). Initially, emergence was recorded at weekly intervals but after 2 mo, because of the decline in emergence, it was recorded at 2-wk intervals. Seedlings were counted and removed until no further emergence was recorded in three consecutive measurements. The counts thus obtained were expressed as cumulative seedling emergence percentage, i.e., percentage of the total emergence. According to the resistance status confirmed in herbicide resistance screening, the populations were grouped under three resistance classes, i.e., ACCase-inhibiting herbicide resistant (ACC-R), ALS-inhibiting herbicide resistant (ALS-R), and susceptible. Cumulative seedling emergence in these different groups at each sampling time (days after sowing [DAS]) was compared using ANOVA. Cumulative seedling emergence values were fitted to a functional three-parameter sigmoid model with the use of SigmaPlot version 12.5. The model fitted was

$$E(\%) = E_{\max}/\{1 + exp - [(x - T_{50})/E_{\text{rate}}]\} \quad [1]$$

where E is the cumulative seedling emergence (%) at time x, $E_{\max}$ is the maximum seedling emergence (%), T_{50} is the time (d) to reach 50% of maximum seedling emergence, and E_{rate} indicates the slope around T_{50}.

Seed-Bank Persistence. To estimate initial seed viability, 20 seeds were randomly selected from each population and tested for viability. For residual seedbank viability, the smooth barley seeds that failed to germinate during the 2013 winter growing season were recovered from the soil (by sieving) in summer and tested for viability. Seed viability was tested with tetrazolium chloride solution (1% w/v). Sterile florets were removed and seeds were soaked in water for 24 h before slicing them longitudinally to expose the embryo and incubating them in 1% w/v tetrazolium chloride solution for another 24 h in the dark at 30 C (Chauhan et al. 2006b). The extent of pink staining observed under a microscope (Stemi 2000®, Carl Ziess, Sydney, Australia) was used as the indicator of viability or nonviability. Seeds with completely stained (pink) embryo were scored as viable, whereas seeds that lacked integrity of embryo and endosperm were considered nonviable or decayed.

To determine seedbank persistence, three independent samples (1 g each) for each population were drawn and total seeds per replicate were counted. The average number of seeds per population thus obtained and percentage of initial seedbank viability were used to calculate the total number of viable seeds sown, which was thus used

Table 1. Herbicide resistance classification of smooth barley populations randomly collected from Upper North (UN) and Eyre Peninsula (EP) regions of South Australia.

Herbicide	Herbicide resistance classification[a] Resistant (> 20% survival)	 Developing resistance (1 to 20% survival)	 Susceptible (0% survival)
	% of populations		
Quizalofop			
Total	7 (6)	8 (7)	85 (77)
UN	17 (4)	22 (5)	61 (14)
EP	3 (2)	3 (2)	94 (63)
Imazamox + Imazapyr			
Total	0	3 (2)	97 (72)
UN	0	0	100 (23)
EP	0	4 (2)	96 (49)
Sulfosulfuron			
Total	0	12 (9)	88 (65)
UN	0	13 (3)	87 (20)
EP	0	12 (6)	88 (45)

[a] Values in parentheses are the number of populations classified in each class.

to calculate seedbank persistence. The formula used was

$$\text{Seedbank persistence}(\%) = (\text{Total number of viable seeds recovered}/\text{Total number of viable seeds sown}) \times 100 \quad [2]$$

To determine a relationship between seedbank persistence (%) and emergence (%), the data were fitted to a functional two-parameter logarithmic model with the use of SigmaPlot version 12.5. The model fitted was

$$y = y_0 + a \times \ln(x) \quad [3]$$

where y is the seedbank persistence (%) at emergence (%) x, and y_0 and a are constants.

Results and Discussion

Smooth barley was found to infest many, but not all cropping fields across the survey area in the UN and EP regions of South Australia. In total, 111 fields were surveyed throughout UN and EP regions, of which 78% were infested with smooth barley and 90 samples had sufficient quantity of seed to allow herbicide resistance screening. Such a high level of occurrence of smooth barley is consistent with the findings of the previous grower survey in which this weed species was rated in the top five most problematic weeds in this region (Fleet and Gill 2010). The majority of surveyed fields had been used to grow wheat (*Triticum aestivum* L.) (62%), barley (*Hordeum vulgare* L.) (12%), peas (*Pisum sativum* L.) (2%), lupins (*Lupinus angustifolius* L.) (2%), canola (*Brassica napus* L.) (1%), and oats (*Avena sativa* L.) (1%). Dominance of cereal crops is consistent with their ability to grow well in low-rainfall environments. Overall, 80% of the collected smooth barley populations came from crop and 20% from the pastures.

Resistance to ACCase-Inhibiting Herbicides. Data were pooled over the two screening experiments and are presented in Table 1. Screening of smooth barley populations randomly collected in the UN and EP regions revealed that the greatest incidence of resistance was to APP herbicide quizalofop, although the overall resistance was low in these regions. Of the 90 smooth barley populations tested for resistance to quizalofop, 13 (15%) populations exhibited some level of resistance and 77 (85%) populations were susceptible. Considerable variation in resistance to ACCase-inhibiting herbicides was identified between the regions. The greatest frequency of quizalofop-resistant populations was observed in the UN region (39%), whereas the frequency of resistance observed in EP was much lower (6%). Overall, 6 (7%) of the tested populations were classified as resistant (> 20% survival), 7 (8%) as developing resistance (1 to 20% survival), and 77 (86%) were susceptible. All resistant populations had greater than 90% survival, whereas plant survival in the populations classified as developing resistance ranged from 2 to 13% at the recommended field rate of quizalofop. This level of survival in the "developing resistance" category is a concern, as anything less than 90% mortality after herbicide treatment is usually regarded as a commercial failure by the growers (Llewellyn and Powles 2001). The majority of the quizalofop-resistant smooth barley populations were collected from wheat fields ($n = 7$, 54%), but resistance was also detected in samples collected from peas ($n = 2$, 15%), barley ($n = 1$, 8%), and pasture ($n = 3$, 23%). Greater frequency of resistance detected in wheat crops appears to be simply related to the dominance of wheat in these cropping regions. Such high levels of survival (> 90%) in resistant populations indicates repeated use of quizalofop and other ACCase-inhibiting herbicides over many years for the control of grass weeds in the UN and EP regions of South Australia. A survey of the cropping region of Tasmania in 2010 identified just one hare barley population with resistance to APP herbicide haloxyfop (Broster et al. 2012). In contrast, surveys conducted in southern New South Wales in 2007 and Western Australia in 2005 found no resistance to ACCase-inhibiting herbicides in smooth and hare barley populations (Broster et al. 2010; Owen et al. 2012).

Resistance to ACCase-inhibiting herbicides has been reported in 46 grass weed species around the world (Heap 2015). In the majority of cases, target-site point mutations have been reported to confer resistance to ACCase-inhibiting herbicides, with metabolism-based resistance less common (Beckie et al. 2012; Délye 2005; Malone et al. 2014). In the case of smooth barley, ACCase-inhibiting herbicide resistance due to target-site point mutations has been recently reported in three populations from South Australia (Shergill et al. 2015). Therefore, the mechanism(s) responsible for ACCase-inhibiting herbicide resistance in smooth barley populations most likely involves an altered target site(s). However, increased detoxification of APP herbicides has also been previously reported as a mechanism of herbicide resistance in hare barley from South Australia (Matthews et al. 2000).

Resistance to ALS-Inhibiting Herbicides. The survey revealed that resistance to ALS-inhibiting herbicides is still rare. Of the 74 smooth barley populations tested for resistance to imidazolinone herbicide mixture imazamox + imazapyr, only 2 (3%) populations were classified as developing resistance, and none of the populations were classified as resistant (Table 1). Both of these two populations were collected from the EP region; none of the populations from the UN had detectable level of resistance to ALS-inhibiting herbicides. Overall, 97% of the populations were susceptible to imazamox + imazapyr, with 100% susceptibility in the UN region and 94% in the EP. The populations classified as developing resistance to imazamox + imazapyr herbicide were collected from wheat fields and had plant survival ranging from 4 to 7% at the recommended field rate. In contrast, imazamox-resistant hare barley from Western Australia showed no plant mortality at the field rate of this herbicide (Owen et al. 2012).

The sulfonylurea herbicide sulfosulfuron was less effective in controlling smooth barley populations as compared with imazamox + imazapyr, but none of the populations were highly resistant (> 20% survival). However, nine populations (12%) were classified as developing resistance, with three (13%) from the UN region and six (12%) from EP. All the populations that survived sulfosulfuron application had a low level of survival ranging from 4 to 11% at the recommended field rate. Of the nine smooth barley populations classified as developing resistance to sulfosulfuron, seven populations were collected from wheat fields and one each from barley and pasture fields. In these low-rainfall cropping districts, because of lack of suitable alternative crop species, cereal-based crop rotations are most common, but they are prone to grass weed infestation (Fleet and Gill 2010). Although resistance to ALS-inhibiting herbicides is the most common form of resistance in weed populations (both monocots and dicots) across the globe (Heap 2015), it appears to be still relatively uncommon in Australia in hare barley, with only two cases reported so far (Owen et al. 2012; Yu et al. 2007).

Multiple and Cross-Resistance. Of the total populations screened with ACCase- and ALS-inhibiting herbicides, none of the populations exhibited multiple resistance. However, two ALS-inhibiting herbicide-resistant populations were found to be resistant to both imazamox + imazapyr and sulfosulfuron herbicides. Sulfonylurea-to-imidazolinone cross-resistance has previously been documented in hare barley populations from western Australia (Owen et al. 2012) and in rigid ryegrass populations from southern Australia (Preston and Powles 2002).

Herbicide resistance screening of smooth barley populations showed a greater incidence of resistance to ACCase-inhibiting herbicides compared with ALS-inhibiting herbicides. Higher levels of resistance to ACCase-inhibiting herbicides compared with ALS-inhibiting herbicides has also been reported in a survey of Italian ryegrass (*Lolium multiflorum* Lam.) in northern Idaho and eastern Washington, as a result of the greater use of ACCase-inhibiting herbicides in those regions (Rauch et al. 2010). Information from the current

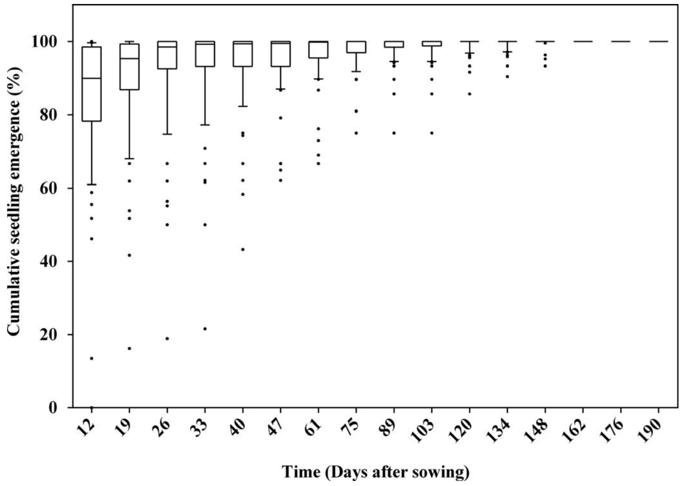

Figure 2. Box and whiskers plot of cumulative seedling emergence percentage of randomly collected smooth barley populations ($n = 63$) measured at different times. Lower and upper boxes represent the second and third quartiles, respectively. Line in the box represents median value. Lower and upper whiskers extend to the 10th and 90th percentiles of the data, respectively.

survey and weed surveys in Tasmania, southern New South Wales (Broster et al. 2012; 2010), and Western Australia (Owen et al. 2012) suggests that herbicide resistance in barley grass is still in early stages of development and weed control with these two modes of action is likely to be effective in the majority of the populations. However, the UN region appears to be a "hot spot" for resistance to ACCase-inhibiting herbicides, with 39% of the populations showing resistance. Growers in those regions need to reconsider their heavy reliance on these herbicides as well as develop strategies to minimize the risk of spread of resistant seeds to the fields that are still susceptible.

Seedling Recruitment. The distribution of cumulative seedling emergence percentage of 63 smooth barley populations measured at different times (DAS) is shown in box and whiskers plot in Figure 2. The majority (75%) of smooth barley populations emerged rapidly and reached cumulative seedling emergence of 87 to 100% within 19 DAS. Even at the first count (12 DAS), nearly half of the populations had reached 78 to 99% cumulative seedling emergence. The susceptible and non-dormant check population, Yaninee, also exhibited rapid emergence ($T_{50} = 8$d); short-lived innate dormancy and rapid emergence after autumn rains is a typical behavior of smooth and hare barley reported in the Australian literature (Cocks and Donald 1973; Peltzer and Matson 2002; Smith 1968). However, several populations continued to exhibit some seedling emergence even as late as 148 DAS. It is likely that the populations that exhibited delayed emergence possessed greater dormancy compared with the populations with rapid emergence. Similar large variation in seedling emergence between smooth barley populations has been recently reported by Fleet and Gill (2012). They reported that some populations from cropping fields had developed dormancy mechanisms to delay emergence that would allow them to evade presowing weed control in the field. Selection pressure from weed-control treatments in cropping fields appears to have selected physiological mechanisms that increase expression of seed dormancy in smooth barley (Fleet and Gill 2012).

The T_{50} values obtained by fitting a three-parameter sigmoid model (Equation 1) to the cumulative seedling emergence of different smooth barley populations varied from 6 to 44 d, with a range of 6 to 26 d in the populations from the UN region and 6 to 44 d in the EP populations (Figure 3). The median T_{50} value for seedling emergence (8 d) was very similar for the UN and EP populations (unpaired t test, $P = 0.66$). These results indicate that the majority of smooth barley populations have low seed dormancy and germinate rapidly, which is consistent with the findings of previous research on this species (Cocks and Donald 1973; Peltzer and Matson 2002). However, some smooth barley populations had seven fold greater T_{50} values than others. Higher T_{50} values is an indication of delayed emergence, which is likely to be associated with greater seed dormancy as previously reported in smooth barley by Fleet and Gill (2012). According to Buhler et al. (1997), the knowledge of emergence patterns of weeds could be used to determine optimum timing of cultivation and POST herbicide application. Delaying sowing to allow high weed seed germination and using herbicides to control weed populations has been advocated as an effective weed management tool (Owen et al. 2014). Fleet and Gill (2010) also reported that delaying sowing by 3 wk resulted in 75% reduction in smooth barley infestation in wheat. They further reported that delayed sowing helped dormant smooth barley population to satisfy the cold stratification requirement for germination so they could then be easily

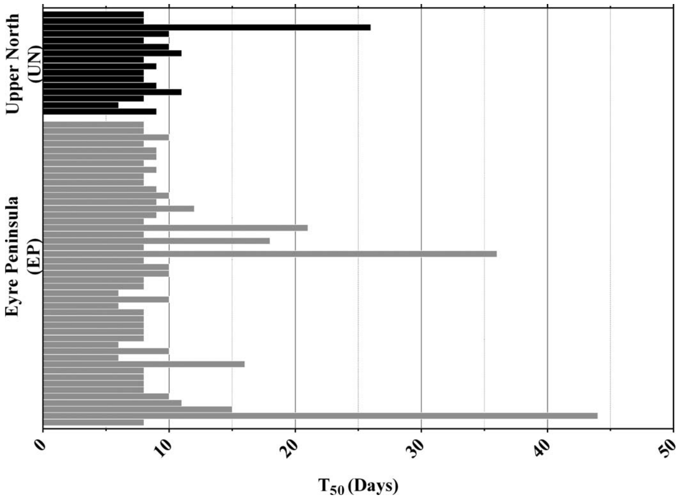

Figure 3. Time (d) taken to reach 50% of maximum seedling emergence (T_{50}) of smooth barley populations randomly collected from the Upper North (UN) and Eyre Peninsula (EP) regions of South Australia. T_{50} values were calculated by fitting cumulative seedling emergence percent data to a functional three-parameter sigmoid model (Equation 1). Each horizontal bar represents T_{50} value for each population ($n = 63$).

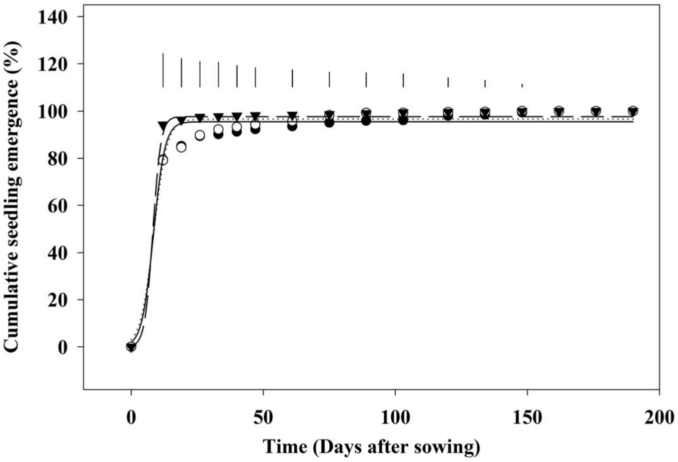

Figure 4. Cumulative seedling emergence pattern of three resistance groups; susceptible (solid line, •, $n = 44$), ACC-R (dotted line, ○, $n = 11$), and ALS-R (dashed line, ▼, $n = 8$) of smooth barley populations measured at different times. Vertical bars represent LSD ($P > 0.05$) according to ANOVA. Lines represent a functional three-parameter sigmoid model (Equation 1) fitted to the mean of cumulative seedling emergence percent data for each at different times.

controlled by PRE herbicides. Similar findings were also reported by Buhler and Gunsolus (1996) in soybean (*Glycine max* L.), where delayed planting reduced weed infestation and improved weed control with rotary hoeing and cultivation. However, delayed sowing can incur a large yield penalty and is generally not preferred by the growers in Australian rain-fed cropping systems (reviewed in Roper et al. 2012). Despite this, delayed sowing could be used occasionally in fields infested with highly dormant weed populations, especially if they are known to be resistant to selective herbicides.

Relationship between Seedling Emergence and Herbicide Resistance. The seedling recruitment data showed that the majority of the herbicide-resistant smooth barley populations were non-dormant and germinated rapidly ($T_{50} < 11$ d). However, there were two ACCase-inhibiting herbicide-resistant populations that had not completely emerged after 47 and 120 DAS. The T_{50} of these two biotypes estimated from the model (Equation 1) were 18 and 26 d, which is much greater than the T_{50} for the non-dormant resistant biotypes (6 to 11 d). The combination of herbicide resistance with high seed dormancy would make it quite difficult to effectively control such weed populations.

The cumulative seedling emergence data for smooth barley populations were grouped under three resistance classes, i.e., ACC-R, ALS-R, and susceptible. There were no significant differences ($P > 0.05$) in the T_{50} for seedling emergence of smooth barley populations of the three resistance classes (Figure 4). These results suggest that there is no linkage between seed dormancy and resistance status in smooth barley. Gill et al. (1996) also reported no major differences in seedling emergence among the ACC-R and ALS-R and susceptible populations of rigid ryegrass. But in later studies, Vila-Aiub et al. (2005) and Owen et al. (2010) showed some differences in germination and emergence responses between ACC-R or ALS-R and susceptible populations.

In the current study, the greater expression of seed dormancy in two ACCase-resistant populations is unlikely to be directly related to resistance alleles, because many other resistant populations had a much lower T_{50} for seedling emergence (Gundel et al. 2008). The co-occurrence of dormancy and herbicide resistance has been attributed to the impact of selection pressure imposed by management practices associated with decades of intensive cropping rather than herbicide resistance *per se* (Owen et al. 2010). Management practices used in crop production (including cultivation and selective

Table 2. Seedbank persistence variability among the populations of smooth barley collected from the Upper North (UN) and Eyre Peninsula (EP) regions of South Australia.

Seedbank persistence interval	Total	UN	EP
%		% of populations[a]	
0–5	49 (31)	44 (7)	51 (24)
5–10	25 (16)	31 (5)	23 (11)
10–15	21 (13)	25 (4)	19 (9)
15–20	5 (3)	0 (0)	6 (3)

[a] Values in parentheses are the number of populations.

and non-selective herbicide use) are likely to favor survival of late-germinating individuals in a weed population and over time the dormant individuals may become the dominant part of the seedbank (Fleet and Gill 2012; Owen et al. 2014). There is little doubt that highly dormant herbicide-resistant smooth barley populations will be difficult to control in crop fields, especially in cereals, where herbicide options are limited.

Seedbank Persistence. Seeds of smooth barley populations that failed to germinate during the 2013 winter growing season were recovered from the soil in summer and tested for viability to determine whether they had decayed or were still viable. The results revealed that the majority of the smooth barley populations had a low level or no seedbank persistence. Of the 63 randomly collected smooth barley populations tested for seedbank persistence, 47 (75%) had a low level of persistence (< 10%) (Table 2). Overall seedbank persistence was not different between the two regions (unpaired *t* test, P = 0.86), with a maximum of 11% in UN and 20% in EP. There were no differences (unpaired *t* test, P = 0.23) between seedbank persistence of resistant and susceptible populations. Sosnoskie et al. (2013) also reported no detectable differences in seedbank persistence between glyphosate-resistant and glyphosate-susceptible Palmer amaranth (*Amaranthus palmeri* S. Wats) seeds, tested between 0 to 36 mo after burial. All the populations ($n = 17$) with high level of persistence (> 10%) were collected from crop fields, which suggests that the selection pressure imposed by weed-control tactics in crops may have selected for greater seedbank persistence in smooth barley. Previous studies have shown that seeds of smooth barley have a short-lived seedbank and very few seeds are likely to be present after 1 yr (Peltzer and Matson 2002; Popay 1981; Powles et al. 1992). In contrast, the results of the present study clearly indicate that some smooth barley populations have adequate seedbank persistence to reinfest crops in the next season.

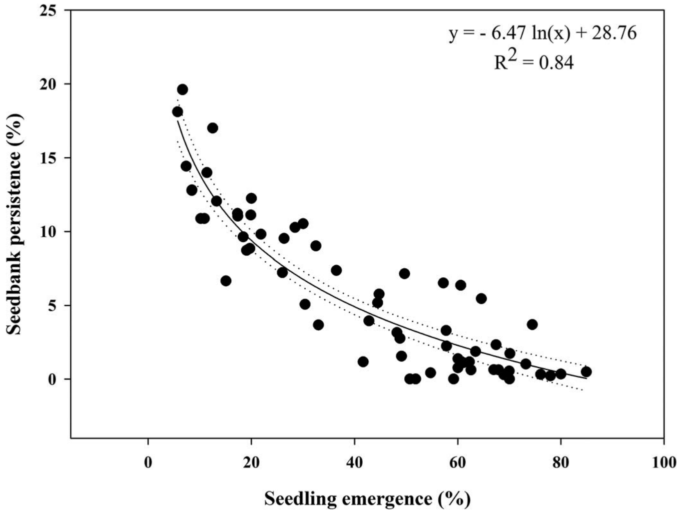

Figure 5. Relationship between seedling emergence (%) and seedbank persistence (%) of smooth barley populations randomly collected from the Upper North (UN) and Eyre Peninsula (EP) regions of South Australia. Lines represent a functional two-parameter logarithmic model fitted (Equation 3) to the seedling emergence (%) and seedbank persistence (%) data for each population ($n = 63$). The band with dotted lines represents the 95% confidence interval.

There was a strong negative relationship between seedling emergence and the level of persistent seedbank of smooth barley populations (Figure 5). Populations that exhibited low seedling recruitment are likely to have a higher level of seed dormancy, which may have enabled greater seedbank persistence. High level of seed decay (50 to 80%) observed in some smooth barley populations was also associated with high seed dormancy. Similar levels of seed decay has been previously reported in other weed species from South Australia. For example, Chauhan et al. (2006a) reported that annual seed decay of rigid ryegrass was > 50% in South Australia cropping systems. Similarly, Kleemann and Gill (2013) reported ~45% seed decay in a population of ripgut brome (*Bromus diandrus* Roth) in South Australia.

In summary, the current study provides valuable insights into the general pattern of herbicide resistance and seedbank behavior of on-farm populations of smooth barley randomly collected from the UN and EP regions of South Australia. It also reports the first known instances of resistance to ALS-inhibiting herbicides in smooth barley. Although the overall occurrence of resistance on farms was low, 39% of the fields in the UN region had detectable level of resistance to the ACCase-inhibiting herbicide quizalofop. Evidence presented suggests that crop management practices used by the growers in the cropping fields has selected for greater seed dormancy and a persistent weed seedbank. The study also reveals that a large proportion of cropping land still contains herbicide-susceptible smooth barley populations, where rotations including ACCase- and ALS-inhibiting herbicides will still provide effective weed control. Additionally, this study found some smooth barley populations that possess ACCase resistance and high seed dormancy. Such populations will be extremely difficult to manage and growers will need to integrate other non-chemical strategies for long-term weed management.

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Literature Cited

Beckie HJ, Warwick SI, Sauder CA (2012) Basis for herbicide resistance in Canadian populations of wild oat (*Avena fatua*). Weed Sci 60:10–18

Broster J, Koetz E, Wu H (2012) Herbicide resistance frequencies in ryegrass (*Lolium* spp.) and other grass species in Tasmania. Plant Prot Q 27:36

Broster JC, Koetz EA, Wu H (2010) A survey of southern New South Wales to determine the level of herbicide resistance in brome grass and barley grass populations. Pages 274–277 *in* Proceedings of the 17th Australasian Weeds Conference. Christchurch, New Zealand: New Zealand Plant Protection Society

Buhler DD, Gunsolus JL (1996) Effect of date of preplant tillage and planting on weed populations and mechanical weed control in soybean (*Glycine max*). Weed Sci 44:373–379

Buhler DD, Hartzler RG, Forcella F (1997) Implications of weed seedbank dynamics to weed management. Weed Sci 45:329–336

Chauhan BS, Gill G, Preston C (2006a) Influence of environmental factors on seed germination and seedling emergence of rigid ryegrass (*Lolium rigidum*). Weed Sci 54:1004–1012

Chauhan BS, Gill G, Preston C (2006b) Influence of tillage systems on vertical distribution, seedling recruitment and persistence of rigid ryegrass (*Lolium rigidum*) seed bank. Weed Sci 54:669–676

Cocks P, Donald C (1973) The germination and establishment of two annual pasture grasses (*Hordeum leporinum* Link and *Lolium rigidum* Gaud.). Aust J Agr Res 24:1–10

Cocks PS, Boyce KG, Kloot PM (1976) The *Hordeum murinum* complex in Australia. Aust J Bot 24:651–62

Davison AW (1971) The ecology of *Hordeum Murinum* L.: II. The Ruderal habit. J Ecol 59:493–506

Délye C (2005) Weed resistance to acetyl coenzyme A carboxylase inhibitors: an update. Weed Sci 53:728–746

Fleet B, Gill G (2010) Barley grass, an emerging weed threat in southern Australian cropping systems. *In* Dove H, Culvenor R, eds. 15th Agronomy Conference. Lincoln, New Zealand: Australian Society of Agronomy

Fleet B, Gill G (2012) Seed dormancy and seedling recruitment in smooth barley (*Hordeum murinum* ssp. *glaucum*) populations in southern Australia. Weed Sci 60:394–400

Ghersa CM, Martínez-Ghersa MA, Brewer TG, Roush ML (1994) Selection pressures for diclofop-methyl resistance and germination time of Italian ryegrass. Agron J 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 Sci 44:252–256

Gundel PE, Martínez-Ghersa MA, Ghersa CM (2008) Dormancy, germination and ageing of *Lolium multiflorum* seeds following contrasting herbicide selection regimes. Eur J Agron 28:606–613

Harris G (1961) The periodicity of germination in some grass species. N Z J Agr Res 4:253–260

Heap I (2015) The international survey of herbicide resistant weeds. www.weedscience.org. Accessed April 8, 2015

Hidayat I (2004) Evolution and Spread of Paraquat-Resistant Barley Grasses *Hordeum glaucum* Steud. and *H. leporinum* Link. Ph.D dissertation: Adelaide, Australia: The University of Adelaide. 176 p

Jasieniuk M, Maxwell BD (1994) Populations genetics and the evolution of herbicide resistance in weeds. Phytoprotection 75:25–35

Kleemann SGL, Gill GS (2013) Seed dormancy and seedling emergence in ripgut brome (*Bromus diandrus*) populations in southern Australia. Weed Sci 61:222–229

Llewellyn RS, Powles SB (2001) High levels of herbicide resistance in rigid ryegrass (*Lolium rigidum*) in the wheat belt of Western Australia. Weed Technol 15:242–248

Malone JM, Boutsalis P, Baker J, Preston C (2014) Distribution of herbicide-resistant acetyl-coenzyme A carboxylase alleles in

Lolium rigidum across grain cropping areas of South Australia. Weed Res 54:78–86

Matthews N, Powles SB, Preston C (2000) Mechanisms of resistance to acetyl-coenzyme A carboxylase inhibiting herbicides in a *Hordeum leporinum* population. Pest Manag Sci 56:441–447

Maxwell BD, Mortimer AM (1994) Selection for herbicide resistance. Pages 1–26 *in* Powles SB, Holtum JAM, eds. Herbicide Resistance in Plants: Biology and Biochemistry. Boca Raton, FL: Lewis Publishers

Owen MJ, Goggin DE, Powles SB (2012) Identification of resistance to either paraquat or ALS-inhibiting herbicides in two Western Australian *Hordeum leporinum* biotypes. Pest Manag Sci 68:757–763

Owen MJ, Goggin DE, Powles SB (2015) Intensive cropping systems select for greater seed dormancy and increased herbicide resistance levels in *Lolium rigidum* (annual ryegrass). Pest Manag Sci 71:966–971

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 Res 51:133–141

Peltzer SC, Matson PT (2002) How fast do the seedbanks of five annual cropping weeds deplete in the absence of weed seed input? Pages 553–555 *in* Proceedings of the 13th Australian Weeds Conference. Perth, Western Australia: Plant Protection Society of WA Inc

Popay A (1981) Germination of seeds of five annual species of barley grass. J Appl Ecol:547–558

Powles SB (1986) Appearance of a biotype of the weed, *Hordeum glaucum* Steud., resistant to the herbicide paraquat. Weed Res 26:167–172

Powles SB, Tucker ES, Morgan TR (1992) Eradication of paraquat-resistant *Hordeum glaucum* Steud. by prevention of seed production for 3 years. Weed Res 32:207–211

Pratley J (1995) Long-term investigations of the effect of tillage practices on crop production at Wagga Wagga, New South Wales. Aust J Exp Agric 35:885–892

Preston C, Holtum JAM, Powles SB (1992) On the mechanism of resistance to paraquat in *Hordeum glaucum* and *H. leporinum*—delayed inhibition of photosynthetic O_2 evolution after paraquat application. Plant Physiol 100:630–636

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

Rauch TA, Thill DC, Gersdorf SA, Price WJ (2010) Widespread occurrence of herbicide-resistant Italian ryegrass (*Lolium multiflorum*) in Northern Idaho and Eastern Washington. Weed Technol 24:281–288

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 Sci Technol 35:32–47

Roper MM, Milroy SP, Poole ML (2012) Green and brown manures in dryland wheat production systems in Mediterranean-type environments. Pages 275–313 (Chapter 5) *in* Donald LS, ed. Advances in Agronomy. San Diego, USA: Academic Press

Shergill LS, Malone J, Boutsalis P, Preston C, Gill GS (2015) Target-site point mutations conferring resistance to ACCase-inhibiting herbicides in smooth barley (*Hordeum glaucum*) and hare barley (*Hordeum leporinum*). Weed Sci 63:408–415

Shergill LS, Preston C, Boutsalis P, Malone J, Gill G (2014) Amino acid substitutions in ACCase gene of barley grass (*Hordeum glaucum* Steud.) associated with resistance to ACCase-inhibiting herbicides. Pages 7–10 *in* Proceedings of 19th Australasian Weeds Conference. Hobart, Tasmania, Australia: Tasmanian Weed Society

Smith DF (1968) The growth of barley grass (*Hordeum leporinum*) in annual pasture. 1. Germination and establishment in comparison with other annual pasture species. Aust J Exp Agric Anim Husb 8:478–483

Sosnoskie LM, Webster TM, Culpepper AS (2013) Glyphosate resistance does not affect Palmer amaranth (*Amaranthus palmeri*) seedbank longevity. Weed Sci 61:283–288

Tranel D, Dekker J (2002) Differential seed germinability in triazine-resistant and -susceptible giant foxtail (*Setaria faberii*). Asian J Plant Sci 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. J Appl Ecol 42:288–298

Walsh MJ, Powles SB (2007) Management strategies for herbicide-resistant weed populations in Australian dryland crop production systems. Weed Technol 21:332–338

Yu Q, Nelson JK, Zheng MQ, Jackson M, Powles SB (2007) Molecular characterisation of resistance to ALS-inhibiting herbicides in *Hordeum leporinum* biotypes. Pest Manag Sci 63:918–27

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421

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