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Limited effect of seed dormancy on the efficacy of preemergence herbicides in rigid ryegrass (*Lolium rigidum*)

Danica E. Goggin[1], Mechelle J. Owen[2], Roberto Busi[3,4] and Ken Flower[5]

[1]Research Associate, Australian Herbicide Resistance Initiative, UWA School of Agriculture and Environment, University of Western Australia, Crawley, WA, Australia; [2]Senior Research Officer, Australian Herbicide Resistance Initiative, UWA School of Agriculture and Environment, University of Western Australia, Crawley, WA, Australia; [3]Research Fellow, Australian Herbicide Resistance Initiative, UWA School of Agriculture and Environment, University of Western Australia, Crawley, WA, Australia; [4]current: Department of Primary Industries and Regional Development, Perth, WA, Australia and [5]Professor, Australian Herbicide Resistance Initiative, UWA School of Agriculture and Environment, University of Western Australia, Crawley, WA, Australia

Research Article

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Corresponding author:
Danica E. Goggin; Email: danica.goggin@uwa.edu.au

Abstract

Widespread resistance to selective postemergence herbicides has led to increased use of preemergence herbicides to control rigid ryegrass (*Lolium rigidum* Gaudin), the major weed of southern Australian cropping systems. Seeds of *L. rigidum* are dormant at maturity, leading to staggered germination across the growing season and avoidance of pre-sowing knockdown herbicides by the later-germinating cohorts. Although it is well known that this selects for higher seed dormancy in intensively cropped areas, there is less information on whether dormant seeds respond differently to preemergence herbicides applied at sowing. To address this, seeds of field-collected *L. rigidum* populations were divided into dormant and nondormant (afterripened) subsamples and treated with sublethal rates of three preemergence herbicides in order to monitor seedling emergence and seed persistence over 6 mo. The presence of prosulfocarb and pyroxasulfone eliminated the nearly 4-fold increase in seedling emergence that typically results from afterripening, while trifluralin was partially inhibitory. In all treatments, the proportion of viable seeds remaining in the soil after 6 mo was negligible (≤3% of the viable seeds originally sown) for both the dormant and nondormant seeds. Application of radiolabeled herbicides to soil and seeds showed that the herbicides persisted in the seed tissue for longer than in the bulk soil. Therefore, the presence of dormant *L. rigidum* seeds in the soil seedbank is unlikely to result in cohorts that can avoid preemergence herbicides.

Introduction

Rigid ryegrass (*Lolium rigidum* Gaudin) is a highly competitive winter annual grass species that has become an economically damaging agricultural weed across the world, particularly in regions with a temperate climate (Matzrafi et al. 2021). If left uncontrolled, it can decrease the yield of a cereal crop by up to 80% (Izquierdo et al. 2013) and produce up to 45,000 seeds m^{-2} (Gill 1996), ensuring subsequent crops are also highly infested. Control of *L. rigidum* is made more difficult by the fact that repeated use of herbicides on this highly adaptable, outcrossing species has resulted in the evolution of resistance to many of the commonly used herbicides, with a large number of populations showing resistance to two or more modes of action (Broster et al. 2022; Busi et al. 2021; Vázquez-García et al. 2020). In Australia, *L. rigidum* costs growers around A$196 million yr^{-1} in yield loss, weed control, and resistance management tools (Bajwa et al. 2021).

Seeds of *L. rigidum* are usually dormant at maturity (meaning they are unable to germinate even under optimal conditions) in order to protect the population from fatal germination during any unusual summer rain events that may occur (Steadman et al. 2004). Those populations with low dormancy still have relatively stringent requirements for germination immediately after shedding, namely, alternating temperatures with an amplitude of around 10 C and light–dark cycles with an optimal photoperiod of 12 h (Gramshaw 1972). Seed dormancy in *L. rigidum* has a physiological basis (i.e., it does not involve physical barriers to seedling emergence; Finch-Savage and Leubner-Metzger 2006) and is considered to be non-deep, as most seeds will have germinated within 2 mo, with the remainder generally not persisting in the soil for longer than 16 mo (Narwal et al. 2008). Warm, dry conditions, such as those encountered by *L. rigidum* seeds lying on the soil surface over the summer months, promote the gradual loss of dormancy (a process known as dry afterripening) and a relaxing of germination requirements so that fully afterripened seeds are able to germinate under any conditions as long as there is adequate moisture and temperatures lie between 5 and 35 C (Steadman 2002). Studies in rice (*Oryza*

sativa L.) seeds have shown that during dry afterripening, levels of the dormancy-inducing hormone abscisic acid decrease, while levels of hydrogen peroxide, which stimulates germination, transiently increase (Yuan et al. 2023). The rate of dormancy loss in *L. rigidum* seeds can be accelerated by incubating imbibed seeds in the dark for a few weeks (known as dark stratification), but these seeds retain the requirement for alternating light and temperature to initiate germination (Steadman 2002).

The relatively low overall level of seed dormancy in *L. rigidum* is still sufficient to allow staggered germination of the seedbank throughout the growing season, so that later-germinating cohorts avoid the knockdown herbicides applied before crop sowing and need to be controlled by in-crop selective herbicides (Darmency et al. 2017), which are the most likely herbicides to have a high-level resistance problem (Busi et al. 2021). To overcome this, growers are increasingly turning to preemergence herbicides applied to the soil at the time of crop sowing (Boutsalis et al. 2014). The Australian no-tillage cropping system relies heavily on the dinitroaniline group of microtubule assembly inhibitors (e.g., trifluralin) and the inhibitors of very-long-chain fatty-acid biosynthesis, particularly the thiocarbamates (e.g., prosulfocarb, triallate) and the isoxazolines (e.g., pyroxasulfone) (Broster et al. 2022). These herbicides do not inhibit seed germination per se, but rather inhibit emergence and growth of seedlings once the radicle and shoot tissues have penetrated the seed coat (e.g., Bolwerk et al. 2024).

The efficacy and persistence of soil-applied herbicides represent a balance between their water solubility, capacity to bind to soil organic matter, volatility, and photosensitivity, and this balance is thus greatly dependent on soil type, environmental conditions and agricultural practices (Bedos et al. 2002). Highly volatile preemergence herbicides such as trifluralin (and, to a lesser extent, prosulfocarb) require incorporation into the soil to avoid excessive dissipation from the soil surface (Chauhan et al. 2006). Given the widely varying persistence of trifluralin (3 to 173 d), prosulfocarb (9 to 38 d), and pyroxasulfone (47 to 134 d) observed in field trials (Adamson et al. 2024; Nègre et al. 2006), the presence of dormant weed seeds in the soil seedbank could have implications for the effectiveness of these herbicides on *L. rigidum* populations. Previous studies have investigated the use of germination stimulants (e.g., potassium nitrate, gibberellic acid, karrikinolide; Das and Das 2018; Stevens et al. 2007) to promote uniform weed seedling emergence before herbicide application, with mixed results. In any case, dormant *L. rigidum* seeds are known to be relatively unresponsive to both gibberellic acid and karrikinolide (Goggin et al. 2009; Stevens et al. 2007). However, there is little information on whether dormant weed seeds lying imbibed but ungerminated in the soil are affected by exposure to preemergence herbicides, or whether they are protected by their dormancy and will germinate normally after the herbicides have dissipated. Therefore, this study investigated the response of freshly harvested *L. rigidum* seed populations to preemergence herbicides in both dormant and nondormant (afterripened) subsamples and examined the relative persistence of radiolabeled herbicides in the soil compared with dormant seed tissue.

Materials and Methods

Seed Material

During a 2020 herbicide-resistance survey of *L. rigidum* populations infesting the Western Australian grainbelt, 18 seed samples were collected from different cropping fields (6 from the high-rainfall zone [H], 5 from the medium-rainfall zone [M], and 7 from the low-rainfall zone [L]; Table 1), as described in Owen et al. (2014). Seeds were collected at crop maturity (October to November) and immediately stored at −20 C to maintain dormancy status: these seeds will hereafter be referred to as "fresh." Subsamples of each population were stored at 37 C for 4 mo to alleviate dormancy via dry afterripening: these seeds will hereafter be referred to as "AR" (afterripened). Six control populations with known dormancy and/or herbicide-resistance status were included in the study (Table 1), three of which (S, R-P4, and pr7) had already been afterripened before the commencement of the study. Nevertheless, subsamples of all six control populations were incubated at 37 C along with the field-collected samples.

Seedling Germination and Growth on Herbicide-impregnated Agar

Both fresh and AR seeds were sown on 0.6% agar-water containing discriminating concentrations of trifluralin (10 µM), prosulfocarb (40 µM), or pyroxasulfone (75 nM), as determined from pilot dose–response studies on the control populations (data not shown). Seed germination and the length of seedling coleoptiles and radicles were measured at 7, 14, and 21 d after the start of imbibition, with seeds being considered to have germinated if either their coleoptile or radicle was >1 mm in length. At 21 d, the viability of ungerminated seeds was assessed using the tetrazolium test (Steadman 2002). Briefly, the seed coat was punctured with a scalpel blade (avoiding the embryo beneath), and seeds were incubated in 1% 2,3,5-triphenyltetrazolium chloride at 30 C in the dark for 24 h. Seeds were then dissected and inspected under a magnifying glass; a uniformly red embryo and aleurone layer indicated a viable seed. There were three replicates of 10 seeds for each herbicide treatment, along with untreated controls, for each fresh and AR seed sample.

Seedling Emergence and Growth in Herbicide-treated Soil

Thirty-cell seedling trays (30 by 20 by 5 cm) were filled with moist low–organic matter potting mix (80% washed river sand, 15% composted pine bark, 5% peat moss), and 25 seeds were sown on the surface of each cell. There were three replicates of each herbicide treatment, plus untreated controls, for each fresh and AR seed sample. The prepared trays and seeds were sprayed with rates of trifluralin, prosulfocarb, or pyroxasulfone corresponding to twice the reported LD_{50} (dose causing 50% mortality) of the susceptible control population (Busi et al. 2017), using a cabinet sprayer equipped with a TeeJet® XR11001 flat-fan dual nozzle (Spraying Systems, Wheaton, IL, USA) delivering herbicide in water at 106 L ha^{-1} at a boom speed of 3.6 km h^{-1} (Owen et al. 2014). Herbicide doses and formulations were as follows: trifluralin, 118 g ha^{-1} (TriflurX, 480 g ai L^{-1}, Nufarm, Laverton North, Australia); prosulfocarb, 556 g ha^{-1} (Boxer, 800 g ai L^{-1}, Bayer Crop Science, Melbourne, Australia); pyroxasulfone, 50 g ha^{-1} (Sakura, 850 g ai kg^{-1}, Bayer Crop Science, Melbourne, Australia). Herbicide rates that were below the recommended field rates but sufficient to kill most of the susceptible population were used in order to increase the likelihood that none of the AR populations would show zero seedling emergence, so that comparisons of AR seedling emergence rates with their dormant counterparts could be made. Following spraying, seeds were lightly covered with fresh potting mix and gently watered. Trays were maintained in a naturally lit glasshouse from the time of spraying

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Table 1. *Lolium rigidum.* populations used for dormancy and herbicide-resistance analysis.

Population[a]	Collection site	Coordinates	Dormancy	Resistance	Reference
H4/2	Kojonup	33.8310°S, 117.1590°E	Unknown	Unknown	—
H4/8	Moodiarrup	33.6120°S, 116.7580°E	Unknown	Unknown	—
H4/26	Darkan	33.3345°S, 116.7331°E	Unknown	Unknown	—
H4/36	Williams	33.0278°S, 116.8792°E	Unknown	Unknown	—
H4/37	Darkan	33.3345°S, 116.7331°E	Unknown	Unknown	—
H4/41	Darkan	33.3345°S, 116.7331°E	Unknown	Unknown	—
M4/12	Kondinin	32.4936°S, 118.2665°E	Unknown	Unknown	—
M4/39	Wagin	33.3050°S, 117.3444°E	Unknown	Unknown	—
M4/46	Katanning	33.6894°S, 117.5551°E	Unknown	Unknown	—
M4/55	Nyabing	33.5412°S, 118.1466°E	Unknown	Unknown	—
M4/59	Lake Grace	33.1009°S, 118.4642°E	Unknown	Unknown	—
L3/1	Kellerberrin	31.6327°S, 117.7193°E	Unknown	Unknown	—
L3/14	Bullfinch	30.9834°S, 119.1119°E	Unknown	Unknown	—
L3/52	Dowerin	31.1976°S, 117.0290°E	Unknown	Unknown	—
L3/53	Dowerin	31.1976°S, 117.0290°E	Unknown	Unknown	—
L3/54	Wyalkatchem	31.1808°S, 117.3819°E	Unknown	Unknown	—
L3/56	Wyalkatchem	31.1808°S, 117.3819°E	Unknown	Unknown	—
L3/59	Kellerberrin	31.6327°S, 117.7193°E	Unknown	Unknown	—
S (control)	Commercial	N/A	None	Susceptible	Neve and Powles (2005)
ND3 (control)	Wongan Hills (Western Australia)	30.82°S, 116.63°E	Low	Diclofop	Goggin et al. (2011)
VD3 (control)	Wongan Hills (Western Australia)	30.82°S, 116.63°E	High	Diclofop	Goggin et al. (2011)
pr9-P1 (control)	Kadina (South Australia)	33.96°S, 137.71°E	Low	Trifluralin, prosulfocarb, pyroxasulfone; selected once with prosulfocarb	Goggin et al. (2024)
R-P4 (control)	York (Western Australia)	31.87°S, 116.77°E	None	Prosulfocarb, pyroxasulfone; selected four times with pyroxasulfone	Goggin et al. (2024)
pr7 (control)	Kadina (South Australia)	33.96°S, 137.71°E	None	Trifluralin	Goggin et al. (2024)

[a]Populations H4/2 to L3/59 were collected from the Western Australian grainbelt at harvest in 2020. Control populations of known dormancy or resistance status were also included in the study.

in April until the conclusion of the study in November, with an average mean day/night temperature of 22/15 C. Soil was kept moist by watering from the top, as required, and seedling emergence was counted every 7 d for 182 d. Surviving seedlings (those reaching the 3-leaf stage and beyond) were counted every 28 d. At 168 d, the soil was allowed to start drying out, and after the final count at 182 d, the soil was sieved to retrieve ungerminated seeds. These were sown on agar containing 50 µM fluridone, a treatment known to efficiently stimulate germination in *L. rigidum* seeds (Goggin et al. 2009), and incubated in the dark at room temperature for 21 d to also allow dormancy release via dark stratification (Steadman 2004). Seeds were then transferred to a growth cabinet set at 25/15 C (day/night) with a 12-h photoperiod of white LED light (200 µmol m^{-2} s^{-1}) for a further 21 d. At the end of this time, germination was counted, and the viability of ungerminated seeds was assessed using the tetrazolium test.

Persistence of Radiolabeled Herbicides in Soil and Seeds

To compare the persistence of trifluralin and prosulfocarb in bulk soil compared with seeds, the seeds of the highly dormant population H4/36 were treated with ^{14}C-labeled trifluralin (ring-^{14}C-U, 16.4 mCi $mmol^{-1}$, American Radiolabeled Chemicals, St Louis, MO, USA) or prosulfocarb (ring-^{14}C-U, 48.4 mCi $mmol^{-1}$, Institute of Isotopes, Budapest, Hungary) and placed in soil that had also been treated with the same radiolabeled herbicide. A population with high dormancy was used to minimize the chances that the seeds would germinate before they could be sampled for ^{14}C measurement. The system for each experimental unit was set up with 0.5 g (dry mass) of soil and five seeds placed in the metal lid (area: 1.8 cm^2) of a 30-ml glass vial. The inside of the vial itself had been coated with a slurry of activated charcoal in 10% $CaSO_4$ and allowed to dry, creating a charcoal trap for volatilized herbicide (Savage and Barrentine 1969). Low–organic matter potting mix was treated with a mixture of formulated herbicide at the same rate as was used in the germination test described earlier (118 g ha^{-1} trifluralin or 556 g ha^{-1} prosulfocarb) and radiolabeled herbicide, such that 670 Bq was applied to each individual sample in a total solution volume of 375 µl. Before placement in the potting mix, each seed was treated with a 2-µl droplet of the same formulated herbicide solution containing 135-Bq radiolabeled herbicide per droplet, and allowed to dry. The treated seeds and potting mix were then lightly covered with moist untreated potting mix, and the charcoal trap was attached and sealed with Parafilm. Vials were incubated outside under natural light (starting in early May) and sampled at 0, 7, 28, 84, and 168 d. There were three replicates for each time point and herbicide treatment.

At each time point, radiolabeled herbicides were extracted from the potting mix, seed surfaces, and charcoal traps using a method modified from Johnstone et al. (1998). The seeds in each vial were removed from the potting mix and placed into a separate tube before extraction. Each sample was moistened with 800 µl water and then extracted in 2.2 ml methanol by shaking at room temperature for 60 min. After 30 min of settling, 1.5 ml of supernatant was collected and added to 3 ml water plus 1 ml saturated NaCl. This was partitioned against 3 ml of hexane, and after phase separation, 2.5 ml of the upper (organic) phase was placed into a 6-ml scintillation vial and allowed to evaporate in a fume hood overnight. Samples of the aqueous phase (1 ml) were

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also collected for scintillation counting in case polar herbicide metabolites were produced by soil microbes or the seed coat during incubation. Surface-extracted seeds were ground in 500 µl 0.5 M KOH in 50% (v/v) methanol, and the homogenate was placed directly in a scintillation vial, along with a 500-µl rinse of the mortar and pestle. It should be noted that this extraction method, while enhancing recovery of herbicide that might be bound to insoluble seed material, does not allow the applied herbicides and their potential degradation products to be distinguished on the basis of polarity. All samples were mixed with Ultima Gold XR scintillation cocktail (Revvity, Melbourne, Australia) and counted in a TriCarb 4190 TR liquid scintillation counter (Revvity, Boston, MA, USA).

Data Analysis

Seed viability (agar study) or fate (soil study: seeds were classified as emerged+survived, emerged+died, ungerminated+alive, or dead) at the end of each experiment was expressed as a percentage of the total viable seeds sown. Germination or emergence over each 7-d interval was calculated as a cumulative percentage of total viable seeds in the sample. Weighted germination index, in which the earliest-germinating seeds are given the highest weight and later-germinating seeds are given progressively less weight, was used as measure of seed dormancy and was calculated according to the formula in Benech-Arnold et al. (1991):

$$\text{Germination index} = (26 \times n_1) + (25 \times n_2) + \ldots + (1 \times n_{26}) \quad [1]$$

where $n_1, n_2, \ldots, n_{26}$ is the number of seeds germinated in each week from weeks 1 to 26, and 26, 25, . . . , 1 are the weights given to the number of seeds germinated in each week. In the agar study, coleoptile and radicle elongation of herbicide-treated seedlings was expressed as a percentage of the elongation of untreated seedlings. To trace the fate of ^{14}C-labeled herbicides applied to soil and seed surfaces, the amount of radioactivity present in each sample (soil surface, seed surface, seed interior, charcoal trap) was expressed as a percentage of the total ^{14}C present in the system at $t = 0$.

The effect of herbicide and afterripening treatment on seed viability or fate and on weighted germination index was assessed by two-factor ANOVA following square-root transformation of the data to achieve homoskedasticity (confirmed by Levene's test for homogeneity of variances). Multiple pairwise comparisons were assessed by Tukey's honest significant difference (HSD) test using the R statistical package (v. 4.3.0: R Core Team 2023) ($\alpha = 0.05$). The time taken for the measured ^{14}C in soil and seed samples to reach 50% of initial values (t_{50}) was estimated using logarithmic regression in Excel. The ^{14}C remaining was regressed against the ln of time (an initial time point of 2 h, or 0.0833 d, was used so that the ln at $t = 0$ d could be taken), and the resulting parameters were used to calculate the t_{50}.

Results and Discussion

A high efficacy of preemergence herbicides is essential for crop production in no-tillage cropping systems, such as those in southern Australia. Intensive cropping has been shown to select for increased seed dormancy in weed populations by removing the early-germinating seed cohort with knockdown herbicides (reviewed in Batlla et al. 2020), and so the preemergence herbicides are challenged not only by increasing levels of resistance to their modes of action (Busi et al. 2021), but by higher seed dormancy, which could affect their efficacy in ways that are difficult to predict. In the current study, seeds of *L. rigidum* populations from across the Western Australian cropping region were assessed for their response to the preemergence herbicides trifluralin, prosulfocarb, and pyroxasulfone, in both their freshly harvested, dormant state and their afterripened, less-dormant state.

Preliminary Characterization of Seed Populations

When imbibed on herbicide-free agar, all populations, except those controls that had already been afterripened (S, R-P4 and pr7), showed faster and higher germination of AR compared with fresh seeds ($P = 0.002$), with final germination percentages ranging from 11% to 83% for fresh seeds and 57% to 100% for AR seeds (Supplementary Figure S1). Incubation of seeds on agar containing discriminating concentrations of herbicide resulted in only minor losses of viability (Figure 1A), and only prosulfocarb caused a statistically ($P = 0.012$) and biologically (2-fold) significant decrease in germination index, but only in AR seeds (Figure 1B). This is in line with previous studies on various weed or native species (susceptible to the herbicides in question) treated with trifluralin (e.g., Cooper et al. 2024), prosulfocarb (e.g., Nègre et al. 2006), or pyroxasulfone (e.g., Moore et al. 2021), which demonstrated relatively minor inhibition of germination by these herbicides. Although germination on herbicide-containing agar resulted in significant ($P < 0.001$) decreases in seedling coleoptile and radicle length relative to the untreated controls, there was little difference between the length of seedlings germinated from fresh or AR seeds in the presence of herbicides (Figure 1C and 1D).

The weighted germination index of herbicide-untreated, fresh seeds sown on agar was used as an indicator of relative seed dormancy. As expected, the three control populations that had already been afterripened before the commencement of the study had significantly ($P < 0.001$) higher germination indices (>250) than the populations with freshly harvested seeds (Figure 2; Supplementary Figure S2A). Among the field-collected populations, germination indices ranged from 17 to 154, lower ($P = 0.001$) than nondormant control population ND3 (214) and encompassing that of the dormant control VD3 (49) (Figure 2; Supplementary Figure S2A).

The mean elongation of radicles and coleoptiles of seedlings germinated (from AR seeds) on agar containing discriminating concentrations of trifluralin, prosulfocarb, or pyroxasulfone was used as an indicator of relative resistance. Elongation of field population L3/59 was not significantly different from that of resistant control populations R-P4 and pr9-P1 but was lower ($P < 0.001$) than that of pr7; of the other populations, none had significantly higher elongation than the susceptible control population S (Figure 2; Supplementary Figure S2B). Therefore, taken as a group, the field-collected populations were considered to be dormant and, overall, susceptible to the three herbicides used.

Effect of Afterripening on Seedling Emergence and Survival in Soil

Similar to the results of the short-term experiment on agar, afterripening generally increased the speed and extent of emergence of herbicide-untreated field populations and the control populations that had not already been afterripened (Table 2; Supplementary Figure S3). As a side note, the overall average final emergence of seedlings from AR seeds in soil was 20 percentage points lower than the average final germination on agar (65% vs. 85%), illustrating the difference between the technical definition of germination in a controlled environment

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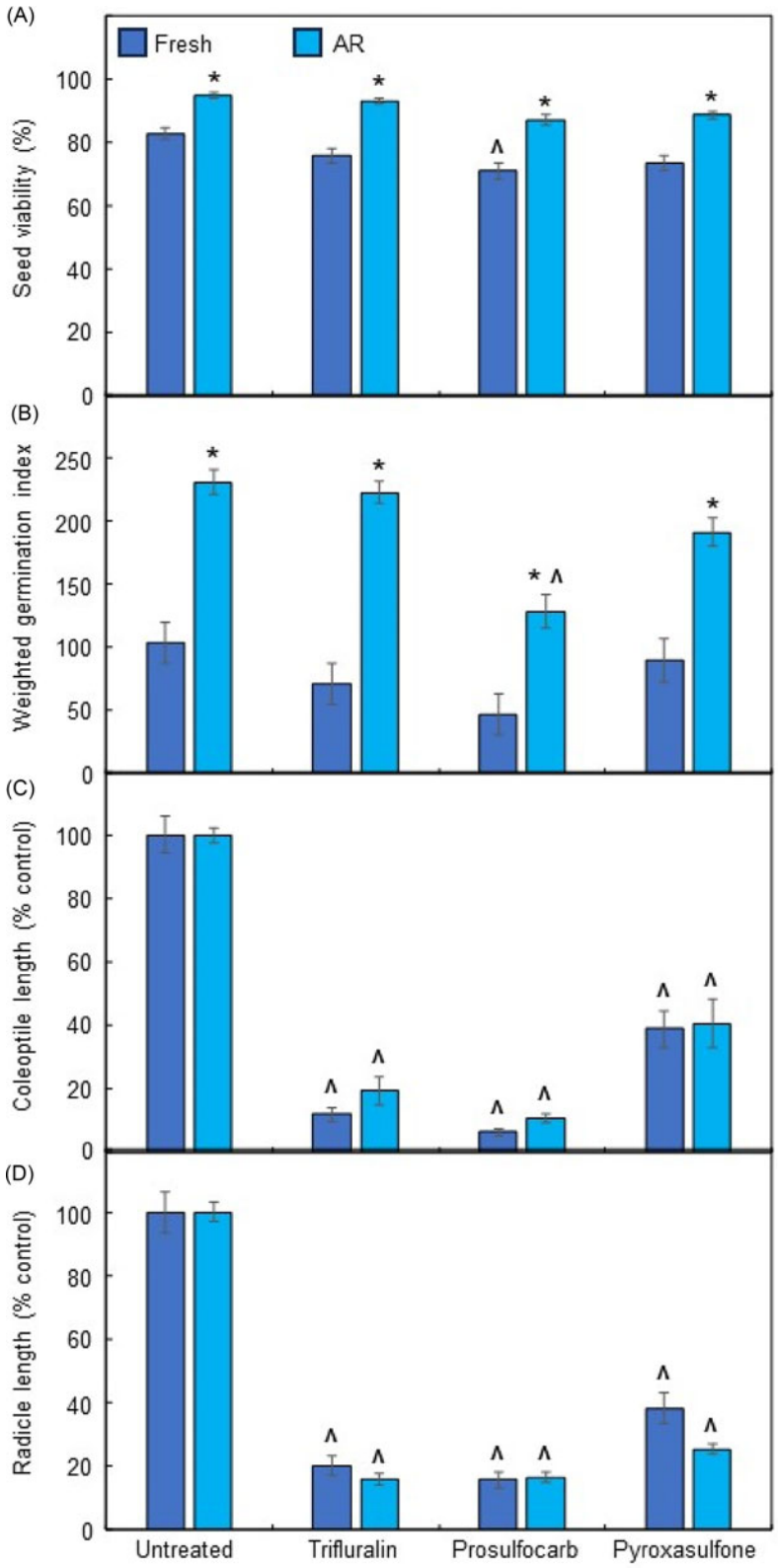

Figure 1. Responses of seeds germinated on herbicide-containing agar. Data for all 24 populations were pooled to assess the effects of herbicides on (A) seed viability, (B) weighted germination index, (C) coleoptile elongation, and (D) radicle elongation of fresh and afterripened (AR) seeds. Data are means ± SE ($n = 24$). Symbols above bars denote significant ($P < 0.05$) differences between fresh and AR seeds within a herbicide treatment (asterisk, *) or differences between herbicide-treated and untreated seeds within the fresh and AR cohorts (caret, ^).

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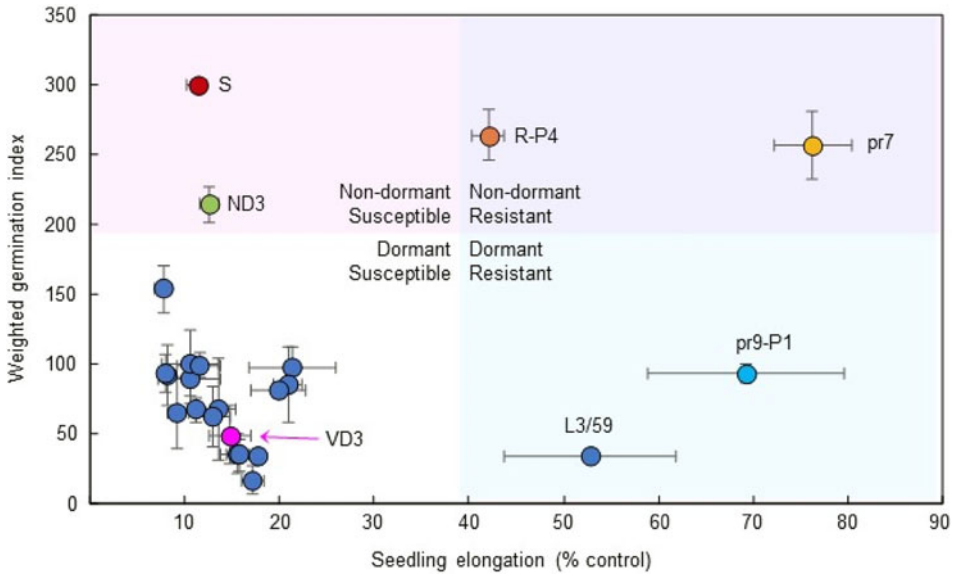

Figure 2. Plot of weighted germination index of untreated fresh seeds vs. seedling elongation of afterripened seeds germinated on herbicide-containing agar to indicate the relative dormancy and herbicide resistance of field-collected populations (dark blue dots) compared with control populations (colored dots). Values are means ± SE ($n = 3$). Shaded areas show the arbitrary dormancy and resistance category into which each population falls.

(penetration of the seed coat by embryo tissues) and the actual emergence of seedlings from soil under more field-like conditions.

The proportion of seedlings that emerged but died before reaching the 3-leaf stage was very low in all treatments (overall average: 2%), as was the proportion of seeds that were ungerminated but still alive after 182 d burial in soil (3%) (Figure 3A), meaning that there were no statistically significant differences among populations or treatments for these parameters. Averaged over all field-collected populations, the mortality of herbicide-untreated fresh seeds was double that of AR seeds; correspondingly, herbicide treatment had no significant effect on the mortality of fresh seeds but increased the proportion of dead seeds in the AR samples by 2- to 3-fold ($P = 0.002$) (Figure 3A). The very low proportion of viable seeds remaining in the soil at the end of the experiment indicates that under these conditions, *L. rigidum* seeds either germinate or die, rather than remaining viable in the soil beyond the current growing season. Similar conclusions were drawn by Narwal et al. (2008) and Sousa-Ortega et al. (2020) in studies that did not involve herbicides but showed that the viable *L. rigidum* seedbank was very low at the end of one growing season irrespective of soil type, rainfall regime, or seed burial depth. It was proposed that the *L. rigidum* seedbank could be effectively eliminated if seed production was prevented for 18 to 24 mo (Narwal et al. 2008).

The low proportion of *L. rigidum* seeds in the current study that emerged but died early means that the weighted germination index (accounting for seedlings that survived to the 3-leaf stage plus those that died soon after emerging) closely parallels the survival data for 3-leaf seedlings. Two-factor ANOVA showed significant ($P < 0.001$) main effects for both herbicide treatment and dormancy status (fresh or AR) and a significant interaction between them (Supplementary Table S1). The weighted germination index of the majority (79%) of populations was unaffected by any herbicide treatment when the seeds were fresh but was significantly ($P = 0.039$) lower than that of the untreated controls for AR seeds (Table 2; Supplementary Figure S4). When the data for the 18 field-collected populations were pooled, the weighted germination index of AR seeds was 4- and 3-fold ($P = 0.002$) higher than that of fresh seeds in the control and trifluralin treatments, respectively, but was not significantly different from that of fresh seeds in the prosulfocarb and pyroxasulfone treatments (Figure 3B). In fresh seeds, herbicides did not have a significant effect on weighted germination index, while in AR seeds, all three herbicides decreased the germination index by ≥50% ($P < 0.001$) relative to the untreated control (Figure 3B). The fact that soil emergence of seedlings from afterripened *L. rigidum* seeds was inhibited by all three herbicides, while emergence from fresh seeds was not, demonstrates that the stimulation of seedling emergence normally afforded by afterripening was cancelled out by the presence of the herbicides (Figure 3B). This was not the case in the agar experiment, where the germination index of afterripened seeds was always higher than that of the corresponding fresh seeds (Figure 1B). This suggests that under controlled, soil-free conditions within a relatively short time frame, the changes in the seed tissues wrought by dry afterripening (potentially, altered hormone and reactive oxygen species balance; Yuan et al. 2023) do not affect their subsequent response to herbicides targeting the cytoskeleton (trifluralin) or cellular membranes (prosulfocarb and pyroxasulfone). However, seeds buried in soil for extended periods are more susceptible to decay and infection due to the presence of soil microbes, and it is known that species with physiological seed dormancy have a greater investment in chemical defenses than those whose seeds lie quiescent in the soil due to a lack of germination cues (analogous to dormant seeds that have been partially afterripened) (reviewed in Dalling et al. 2020). A study on blackgrass (*Alopecurus myosuroides* Huds.), a winter annual grass weed infesting European cropping fields that shares many dormancy and resistance-related characteristics with *L. rigidum*, showed that upon imbibition, expression of several genes related to defense from pathogens, fungi, and bacteria was higher in dormant compared with afterripened seeds (Holloway et al. 2024). Therefore, it is possible that in herbicide-treated soil, afterripened *L. rigidum* seeds lose some or all of their enhanced ability to emerge compared with dormant seeds, due to the combined stresses of (1)

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Table 2. Effect of herbicide and afterripening (AR) treatments on the weighted germination index (WGI) of seeds sown on soil[a]

Effect of herbicide treatment	No. populations	Effect of dormancy status	No. populations
No effect of herbicides on WGI of fresh seeds	19	AR has no effect on WGI of herbicide-untreated seeds	8
All herbicides decrease WGI of fresh seeds	2	AR increases WGI of herbicide-untreated seeds	16
Trifluralin and pyroxasulfone decrease WGI of fresh seeds	1	AR has no effect on WGI of trifluralin-treated seeds	18
Prosulfocarb and pyroxasulfone decrease WGI of fresh seeds	1	AR increases WGI of trifluralin-treated seeds	6
Trifluralin increases WGI of fresh seeds	1	AR has no effect on WGI of prosulfocarb-treated seeds	21
No effect of herbicides on WGI of AR seeds	2	AR increases WGI of prosulfocarb-treated seeds	1
All herbicides decrease WGI of AR seeds	17	AR decreases WGI of prosulfocarb-treated seeds	2
Prosulfocarb and pyroxasulfone decrease WGI of AR seeds	4	AR has no effect on WGI of pyroxasulfone-treated seeds	23
Trifluralin decreases WGI of AR seeds	1	AR decreases WGI of pyroxasulfone-treated seeds	1

[a]Fresh (dormant) and AR seeds of 24 populations were sown onto soil and treated with sublethal rates of trifluralin, prosulfocarb, or pyroxasulfone, and seedling emergence was monitored for 6 mo to calculate WGI. Comparisons of untreated vs. herbicide-treated seeds (within fresh and AR cohorts) and fresh vs. AR seeds (within herbicide treatments) were performed using Tukey's honest significant difference (HSD) test. The number of populations showing significant ($P < 0.05$) differences between treatments are shown.

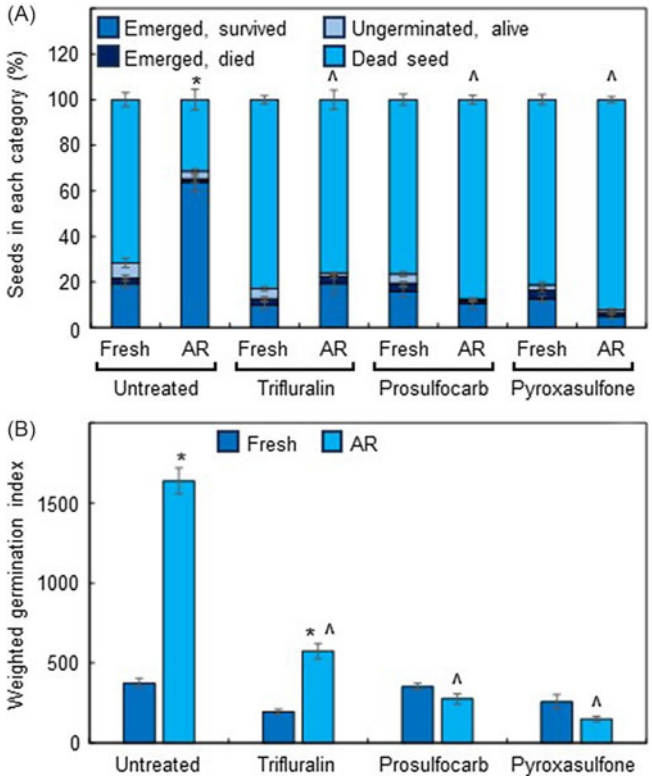

Figure 3. Response of field-collected populations to treatment with sublethal rates of preemergence herbicides. Fresh and afterripened (AR) seeds from each population were sown onto the surface of soil and treated with trifluralin, prosulfocarb, or pyroxasulfone. Seedling emergence and survival were monitored weekly for 6 mo, and the viability of ungerminated seeds was assessed to determine the fate (A) and weighted germination index (B) of the seed populations. Values are means ± SE ($n = 18$); symbols above bars denote significant ($P < 0.05$) differences between fresh and AR seeds within herbicide treatments (asterisk, *) or between herbicide-treated and -untreated seeds within the fresh and AR cohorts (caret, ^).

microbial action before and during early germination and (2) herbicidal effects on establishing seedling tissues. Future studies should therefore examine parameters related to seed defense and damage in dormant and nondormant *L. rigidum* seeds that are placed in sterilized versus non-sterilized, herbicide-treated soils, in order to clarify this point. However, as the current study showed only low percentages of viable seeds remaining in the soil or undergoing fatal germination in both the dormant and afterripened subsamples, the presence of dormant seeds in an *L. rigidum* seedbank is unlikely to result in the failure of trifluralin, prosulfocarb, or pyroxasulfone to the same extent that would be observed if the population had a resistance mechanism.

Persistence of Radiolabeled Herbicides in Soil and Seeds

Another factor that can potentially lead to lower efficacy of preemergence herbicides is their loss of bioavailability, mainly through volatilization or strong binding to soil organic matter (Bedos et al. 2002; Nègre et al. 2006). It should be noted that in the current study, the ^{14}C recovered from the charcoal trap above the treated soil and seeds in both the trifluralin and prosulfocarb systems was negligible across the entire experiment (Figure 4A and 4B), and thus the volatilized portion of the herbicides was either lost from the system or irreversibly bound to the charcoal, vial walls, lid, and/or Parafilm seal (e.g., Carringer et al. 1975; Sharom and Solomon 1981) and could not be accounted for. Nevertheless, there were clear differences in the dissipation of the herbicides from the other samples. Both trifluralin and prosulfocarb disappeared rapidly from the soil and from the seed surfaces, with <40% of the initial ^{14}C in these samples remaining after 7 d of incubation (Figure 4A and 4B). Logarithmic regression indicated that the time taken to reach 50% of the initial ^{14}C-trifluralin in the soil and on the seed surfaces was 2.6 ($P = 0.011$) and 1.9 d ($P = 0.009$), respectively, while 50% of initial [^{14}C]prosulfocarb was dissipated in 4.0 d ($P = 0.005$) from the soil and in 2.5 d ($P = 0.002$) from the seed surfaces. In contrast, the ^{14}C in the interior of the seeds was initially lower (10% to 35% of that in the soil or on the seed surface) but stayed constant over the entire 168 d (trifluralin) or remained above 50% for 84 d (prosulfocarb) (Figure 4A and 4B).

To relate the persistence of herbicide-derived ^{14}C in the experimental system to the emergence of seedlings over the same span of time, the emergence and overall survival (emergence plus viable ungerminated seeds) data for fresh seeds, averaged over the 18 field-collected populations, were plotted underneath the herbicide dissipation data (Figure 4C–F). This illustrates that although emergence of the dormant seeds was only ≤20% over a 168-d span of time, there was almost no viable seedbank remaining at 182 d (Figure 4C–F).

Although seeds in the field may not directly receive herbicide spray droplets if they have been covered with soil or stubble, a proportion of applied herbicide still reaches the seed(ling) tissue, as evidenced by the effectiveness of preemergence herbicides used appropriately in the field (and note that most herbicide labels include warnings that efficacy will be decreased if the stubble layer

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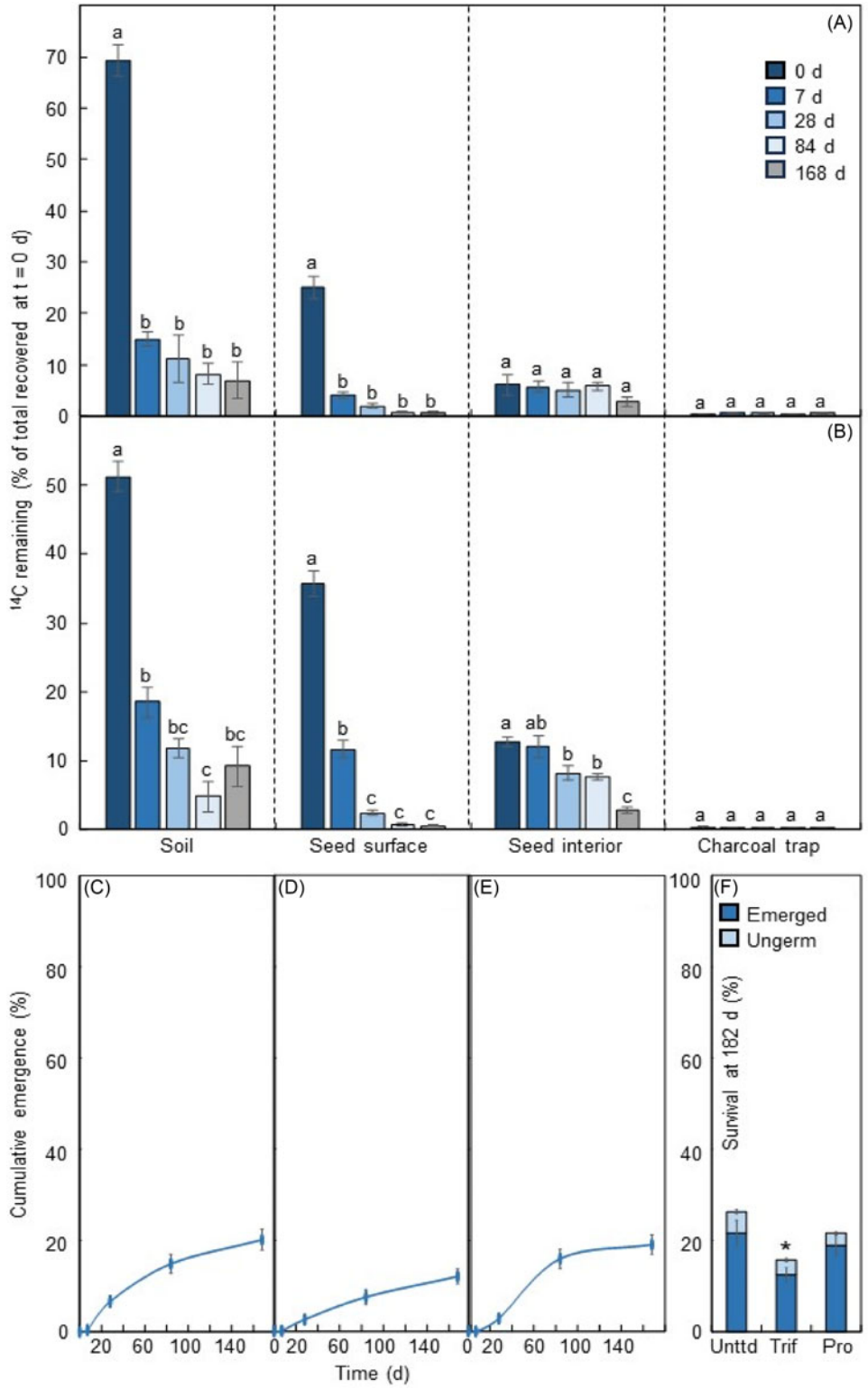

Figure 4. Dissipation of (A) trifluralin and (B) prosulfocarb from soil and the surface and interior of seeds treated with ^{14}C-labeled herbicides and incubated in closed vials with charcoal traps for 168 d. Data are expressed as a percentage of the total ^{14}C recovered from the vial system at $t = 0$ d. Cumulative emergence data pooled across the 18 field-collected populations are shown for (C) untreated, (D) trifluralin-treated, and (E) prosulfocarb-treated fresh seeds at the equivalent time points as those in (A) and (B). Total survival (emergence to the 3-leaf stage plus viable ungerminated seeds) of the pooled field-collected populations at 182 d after treatment with no herbicide (Unttd), trifluralin (Trif), or prosulfocarb (Pro) is shown in (F). In A and B, values are means ± SE ($n = 3$); within each sample type, different letters above bars denote significant ($P < 0.05$) differences among time points. In C–F, values are means ± SE ($n = 18$). In F, an asterisk (*) denotes a significant difference between survival of herbicide-treated and untreated seeds.

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is too heavy). Therefore, dissipation of trifluralin and prosulfocarb, and possibly other preemergence herbicides, from the soil before the entire seed cohort has initiated germination may not be a major problem, because a reservoir is retained in the seed that could act as a continuous slow-release source of herbicide. A limitation of this study is that the harsh solvent employed to extract the ^{14}C from the seed interiors does not permit further analysis of the nature of the radiolabeled compound, so it is unknown whether the ^{14}C represents phytotoxic compounds (i.e. trifluralin and prosulfocarb sulfoxide) or their degradation products and polar metabolites (potentially less phytotoxic). Future work could potentially resolve this problem by using label-free mass spectrometry–based methods to unequivocally detect the parent herbicides and their derivatives. However, trifluralin and triallate (like prosulfocarb, a thiocarbamate herbicide) applied to oat (*Avena sativa* L.) seeds accumulated in the testa and pericarp rather than the embryo (Heath et al. 1984), so it is likely that they remained in their unmetabolized form.

From a practical standpoint, the relatively low level of seed dormancy in *L. rigidum* appears unlikely in itself to negatively affect the efficacy of trifluralin, prosulfocarb, or pyroxasulfone; the higher-dormancy seeds are unable to survive for extended periods in the soil (e.g., Figure 4F), and the herbicides persist long enough in moist soil to inhibit the emergence of the afterripened seeds (Figure 2). More Australian farmers are adopting dry sowing, with incorporation of preemergence herbicides, before the onset of the (increasingly unreliable) season-opening rains, in order to be able to grow large areas of crop within a relatively short season (Fletcher et al. 2015). Persistence of preemergence herbicides in dry soil was previously found to be greater than in wet soil (Minkey 2017), but it is unknown whether the herbicides are as effectively absorbed by the *L. rigidum* seeds themselves when the soil is dry. Therefore, under dry sowing conditions, it may be beneficial to increase crop seed rate to provide greater competition against weeds. The presence of a thick stubble layer can also reduce the efficacy of preemergence herbicides and should be taken into account when deciding on a weed management plan, but the potentially higher moisture level under the stubble could contribute to accelerated dormancy release, allowing the seedbank to germinate earlier (Steadman 2004) and be exposed to a higher herbicide concentration. There have been several studies investigating ways of depleting the seedbank of troublesome weeds, for example, by reducing seed set of parent plants; destroying the seeds at harvest; stimulating uniform germination of the seedbank so that no individuals escape the knockdown herbicides; or inhibiting germination via deep burial or use of cover crops so that no individuals can emerge in the cash crop (e.g., Bajwa et al. 2021; Mia et al. 2023; Oliveira et al. 2020). Preemergence herbicides can play an important role in the latter strategy, so avoiding the development of high-level resistance to these herbicides by using them within an integrated management program is essential in helping to manage the seedbank.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/wsc.2025.10042

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