Research Article

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Fluridone: a combination germination stimulant and herbicide for problem fields?

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Abstract

BACKGROUND: Problem weeds in agriculture, such as *Lolium rigidum* Gaud., owe some of their success to their large and dormant seed banks, which permit germination throughout a crop-growing season. Dormant weed seed banks could be greatly depleted by application of a chemical that stimulates early-season germination and then kills the young seedlings. Fluridone, a phytoene desaturase-inhibiting herbicide that can also break seed dormancy, was assessed for its efficacy in this regard.

RESULTS: The germination of fluridone-treated *Lolium rigidum* seeds was stimulated on soils with low organic matter, and almost 100% seedling mortality was observed, while the treatment was only moderately effective on a high-organic-matter potting mix. Seedlings from wheat, canola, common bean and chickpea seeds sown on fluridone-treated sandy loam were bleached and did not survive, but lupins and field peas grew normally.

CONCLUSION: This proof-of-concept study with fluridone suggests that it may be possible to design safe and effective molecules that act as germination stimulants plus herbicides in a range of crop and soil types: a potentially novel way of utilising herbicides to stimulate seed bank germination and a valuable addition to an integrated weed management system.

Supporting information may be found in the online version of this article.

Keywords: fluridone; germination; *Lolium rigidum*; seed dormancy

1 INTRODUCTION

Agricultural productivity, especially in annual field crops, is adversely affected by the presence of weeds that decrease crop yield and quality.[1] The annual weeds infesting the major field crops often mimic crop ontogeny and thus emerge at the same time as crop seedlings.[2] Control of crop weeds can take a variety of forms, but herbicides are the dominant technology.[1] However, crop weeds have evolved strategies to persist in spite of the annual use of herbicides, such as enzymatic herbicide detoxification or an altered herbicide target site.[3] Seed dormancy, a feature of many annual weed species,[4] can also help weeds persist under cropping conditions. By staggering seedling emergence across the crop-growing season, a proportion of the individuals in a weed population avoids pre- and early post-emergent herbicides or other weed control practices.[4] There is evidence that intensive cropping selects for higher weed seed dormancy because only the early-germinating cohort of a population is removed by weed control measures such as soil cultivation and herbicides applied before or at crop sowing, or by early-season post-emergent herbicides.[5,6]

If the annual weed seed bank in a crop field could be stimulated to germinate early and synchronously (i.e. prior to crop sowing), weeds would be more easily controlled. Chemicals that can stimulate germination of the weed seed bank include the smoke-derived molecule karrikinolide (2*H*-furo[2,3-*c*]pyran-2-one)[7] and the gibberellin-active compound AC94377 [1-(3-chlorophthalimido) cyclohexane carboxamide].[8] Neither of these has advanced to commercial practice, and their use would require subsequent weed control techniques such as herbicide application to remove the germinated weed seedlings. Synthetic strigolactones can stimulate the germination of parasitic weeds, which then die in the absence of a host,[9] but do not themselves cause seedling death. Therefore, the present study investigated the potential use of the phytoene desaturase (PDS)-inhibiting herbicide fluridone {1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone} as a combined germination stimulant/herbicide. Abscisic acid (ABA), the plant hormone responsible for induction of seed dormancy and the inhibition of germination, is synthesised in plants via the cleavage and oxidation of xanthophylls.[10] As a PDS inhibitor, fluridone thus indirectly inhibits ABA synthesis and releases seed dormancy, as has been demonstrated in laboratory experiments on a range of species.[11–13] When applied to developing maize seeds within a narrow timeframe, fluridone can also induce pre-harvest sprouting.[14] Germination of the widespread annual grass weed *Lolium rigidum* Gaud. is readily stimulated by fluridone.[15]

Lolium spp. infest crops on all inhabited continents,[16] and *L. rigidum* is a major weed in Australia, occurring almost ubiquitously in southern Australian cropping fields.[3,17,18] Factors contributing to the success of *L. rigidum* include its high fecundity and genetic

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diversity, enabling it to adapt quickly to local conditions,[18] and the fact that the dormant proportion of the seed population remains viable in the soil seed bank for at least 1 year.[19] Therefore, *L. rigidum* is an excellent target to assess whether fluridone could be used as a tool to reduce the weed seed bank by stimulating synchronous germination of weed seeds and then causing death of the emerged seedlings by tissue bleaching.

2 EXPERIMENTAL METHODS

2.1 Chemicals

All chemicals, including technical-grade fluridone, were obtained from Sigma-Aldrich (Sydney, Australia). Sonar A.S., the commercial aquatic herbicide formulation of fluridone, was supplied by SePRO Corporation (Carmel, IN).

2.2 Seeds

The 2007 collection of dormant *Lolium rigidum* seeds characterised by Goggin *et al.*[15] was used. Collection details are given in supporting information Table S1 for all other weed species used in a screen of fluridone efficacy: *Arctotheca calendula* (L.) Levyns, *Avena barbata* Pott ex Link, *Brassica tournefortii* Gouan, *Bromus diandrus* Roth, *Carrichtera annua* (L.) DC., *Echium plantagineum* Kunze, *Ehrharta calycina* Sm., *Eragrostis curvula* Nees, *Raphanus raphanistrum* L., *Sisymbrium erysimoides* Desf., *S. orientale* L. and *Verbesina encelioides* Benth. & Hook.f. ex A. Gray. It is likely that many of these populations have a history of herbicide exposure as they were collected from roadsides and agricultural research stations, but their specific herbicide resistance status is unknown. To preserve their original dormancy level, all seeds were stored in sealed foil bags at −20 °C immediately after threshing.

2.3 Germination tests under controlled conditions

In the screening experiments on a range of weed species, seeds were imbibed and germinated in 90 mm round petri dishes on agar (10 g L^{-1}) containing 0 or 50 μM technical-grade fluridone (and also 50 μM gibberellic acid where specifically indicated) as described.[15] A growth cabinet with day/night temperatures of 25/15 °C and a 12 h photoperiod of cool white fluorescent light at a fluence rate of 45 μmol m^{-2} s^{-1} over 400–700 nm was used for standard germination experiments. Seed germination was assessed weekly for 42 days (dead or empty seeds, identified by visual inspection and gentle squeezing of the ungerminated seeds at the end of the experiment, were excluded from calculations). Depending on seed size, there were 20–50 seeds per replicate, with four replicates per species.

All subsequent experiments were performed using *L. rigidum* seeds, as this is the major weed of Australian cereal cropping systems. To investigate whether a pulse of fluridone applied to seeds was as effective as continuous treatment delivered via fluridone-containing agar, dormant *L. rigidum* seeds were imbibed for 24 h in the dark in petri dishes containing 10 mL of 0 or 50 μM technical-grade fluridone dissolved in 0.1% (v/v) surfactant (polyoxyethylenesorbitan monolaurate), rinsed thoroughly, sown on fluridone-free agar (in petri dishes), washed river sand (in 110 × 55 × 40 mm punnets) or commercial potting mix (50% composted pine bark, 25% washed river sand, 25% peat moss; also in punnets) and then placed under the same standard germination conditions as described above for the multispecies germination tests. Seed germination was assessed weekly until the experiment was concluded 42 days after the start of imbibition. At 7 days after the start of imbibition, when the shoots of the germinated seedlings were still healthy and turgid and their fresh weight was unaffected by fluridone (data not shown), shoot tissue was sampled for carotenoid measurement in order to quantify the extent of tissue bleaching. There were three replicates of 50 seeds for germination and carotenoid assays.

2.4 Measurement of carotenoids

Carotenoids were extracted and quantified as described.[20] Leaf pigments were extracted in 80% acetone at 4 °C under dim light, and carotenoid concentration was calculated from the absorbance at 470 nm, using an extinction coefficient of 2500 (100 mL g^{-1} cm^{-1}).

2.5 Assessment of fluridone as a combination germination stimulant/herbicide

2.5.1 Pot experiments with mature seeds

Dormant, dry *L. rigidum* seeds were sown onto the surface of soil (see below for details of the soil type used in individual experiments) in 310 × 260 × 40 mm trays (300 seeds per tray) and kept outdoors, protected by fine metal mesh, at the University of Western Australia over the summer to permit the gradual release of dormancy via dry after-ripening that is normally experienced by seeds in this Mediterranean-type environment. Duplicate treatments (with three replicates per duplicate) were established, so that one set of trays could be used for accurate germination counting with weekly removal of germinated seedlings, and one set used visually to monitor plant bleaching and to assess growth/mortality. The infrequent light rain events (<5 mm) over the summer after-ripening period were insufficient to imbibe the seeds or elicit germination.

Two days after a deep watering to simulate the onset of the season-opening autumn rains in mid-April, the trays were sprayed with the commercial aquatic herbicide formulation of fluridone diluted in water at a rate of 367 g ha^{-1} of fluridone, using a cabinet sprayer equipped with a flat-fan dual nozzle (TeeJet XR11001) delivering 110 L water ha^{-1} at 210 kPa. Control trays were sprayed with water. The rate of fluridone was based on the experiments of Miller and Carter,[21] who reported that 300 g ha^{-1} of fluridone effectively controlled weeds in cotton fields. Following spraying, trays were kept moist and the plants were fertilised weekly with commercial liquid fertiliser, starting when the seedlings were 7 days old. At 84 days after spraying, the above-ground portions of the surviving ryegrass plants were harvested and dried at 70 °C for 3 days, and the dry mass was recorded.

In 2012, this experiment was performed using potting mix and river sand. Dry seeds were sown in early December 2011, and the simulated rainfall and the fluridone treatment were applied in mid-April 2012. The experiment in 2013 used a sandy loam topsoil (top 10 cm) collected from a farm near York, Western Australia (31° 53′ S, 116° 46′ E), and seeds were sown in early January 2013 and imbibed and sprayed in mid-April. It was originally intended to monitor germination of the endogenous seed bank (rather than newly sown seed) in response to fluridone, but an experiment carried out in 2012 showed that there was negligible germination in either the presence or absence of fluridone (data not shown); therefore, the sandy loam was sown with *L. rigidum* seeds as was done for the river sand and potting mix. Analysis of organic carbon in the three soil types, using the spectrophotometric dichromate oxidation method of Soon and Abboud,[22] gave the following results (expressed as percentage organic carbon): potting mix 7.6; river sand 0.8; sandy loam 2.2.

During the 2013 experiment, seeds of wheat (*Triticum aestivum* L.), canola (*Brassica napus* L.), narrow-leafed lupin (*Lupinus angustifolius* L.), field pea (*Pisum sativum* L.), common bean (*Phaseolus vulgaris* L.) and chickpea (*Cicer arietinum* L.) (5–12 seeds per species per replicate, depending on seed size) were sown in 15 cm diameter pots of sandy loam and immediately sprayed with fluridone to assess crop susceptibility to this herbicide. Wheat, with its known high susceptibility,[23] was also used as a bioassay for the short-term persistence of fluridone in the sandy loam: seeds were sown at depths of either 1 or 5 cm and at four different time points (immediately before spraying and 7, 14 or 21 days after spraying).

2.5.2 *Field experiment with mature seeds*

Sixteen plots of 2 × 5 m were marked in a field of standing wheat stubble (approximately 2 t ha^{-1}) infested with 100–300 *L. rigidum* seeds m^{-2} on a farm near Quairading, Western Australia (32° 01′ S, 117° 24′ E), and eight plots were sprayed with the commercial formulation of fluridone at 367 g ha^{-1} using a hand boom with a water application rate of 115 L ha^{-1}. Spraying was performed in early April 2013 on a day of no wind with a temperature of 34 °C and a humidity of 30%. The other eight plots were sprayed with water as a control, and no ryegrass seedlings were visible in any of the plots at the time of spraying. Weed seed germination in four control and four treated plots was monitored (with minimal disturbance of the soil) over the next 84 days, and the other eight plots (four control, four treated) were used to measure above-ground weed biomass on day 84.

2.5.3 *Application of fluridone during seed development*

Flowering *L. rigidum* plants were sprayed with commercial fluridone to assess its effect on dormancy induction in developing seeds. Dormant parent seeds were induced to germinate on agar by applying a dormancy-breaking treatment of 21 days of hydration in the dark at 20 °C, and, in early May, seven-day-old seedlings were transplanted into 150 mm pots containing potting mix (two seedlings per pot, ten pots). Plants were grown in a naturally lit glasshouse at the University of Western Australia, were kept segregated from other flowering *L. rigidum* plants to avoid gene flow between populations and were watered and fertilised regularly. In mid-September, when all plants were flowering, five pots were sprayed with fluridone as described for the pot experiments above (the other five pots were sprayed with water) and were replaced in the glasshouse after the leaves were dry. Mature seeds were harvested and threshed by hand in mid-December and immediately stored at −20 °C in sealed foil bags. Seed germination on agar was assessed as above, using 20 replicates of 50 seeds for each treatment. Time to 50% germination was calculated according to Coolbear.[24] Carotenoid concentrations were measured in dry seeds and in the shoots of seven-day-old seedlings. To determine whether bleaching persisted in older plants, seeds from fluridone-sprayed flowers were sown on trays containing potting mix (three replicates of 300 seeds) and placed in the glasshouse where they were kept constantly moist until the experiment was concluded at 60 days after sowing.

2.6 Statistical analysis

Untransformed data were analysed using single-factor ANOVA and the least significant difference (LSD) test at a 5% level of significance.

3 RESULTS

3.1 Effectiveness of fluridone under controlled conditions

Under conditions of alternating light and temperature (25/15 °C), the inclusion of 50 μM fluridone in the agar germination medium stimulated ($P < 0.05$) the germination of *Lolium rigidum*, *Sisymbrium erysimoides*, *S. orientale* and *Bromus diandrus* seeds from a control level of 10–40% to ≥90%, and was also effective in stimulating germination of *Ehrharta calycina* (from 1 to 71%), *Echium plantagineum* (from 6 to 31%), *Raphanus raphanistrum* (from 41 to 59%) and *Avena barbata* (from 6 to 19%) (Fig. 1A). Germination of *R. raphanistrum* and *A. barbata* to ≥90% was achieved by inclusion of 50 μM gibberellic acid (GA_3) with 50 μM fluridone, but additive effects of fluridone and GA_3 were not observed for the other species (data not shown).

Germination of dormant *L. rigidum* seeds imbibed in 50 μM fluridone and then sown on fluridone-free agar, river sand or potting mix under controlled conditions was stimulated ($P < 0.05$) relative to untreated seeds, but fluridone was less effective on potting mix (1.6-fold stimulation of germination) than on agar and river sand (2.6-fold stimulation in both cases) (Fig. 1B). Shoot carotenoid concentration in seven-day-old seedlings that had germinated following fluridone treatment of the seeds was reduced more than threefold in seedlings growing on river sand or potting mix, and there was almost no carotenoid in the agar-grown seedlings (Fig. 1B).

3.2 Assessment of fluridone as a germination stimulant plus herbicide

When placed and after-ripened on potting mix, *L. rigidum* seeds sprayed with commercial fluridone showed a twofold ($P < 0.05$) stimulation of germination in the first 7 days after treatment, but after this time there was no difference in germination between the control and the fluridone-sprayed seeds (Fig. 2A). Seeds were generally slower to germinate on river sand than on potting mix, and the effects of fluridone were initially more marked: germination of fluridone-sprayed seeds was at least threefold higher than that of controls over the first 21 days after treatment (Fig. 2A). After this time, however, there were no differences between treatments. The seeds used for the sandy loam experiment in 2013 experienced 1 month less of summer after-ripening than those used in the 2012 experiments on potting mix and river sand; as a result, the control seeds on sandy loam were more dormant (35% final germination) than those on potting mix and river sand (~70% final germination) (Figs 2A and B). Therefore, the difference in germination (twofold; $P < 0.05$) between the control and fluridone-treated seeds in 2013 was maintained throughout the experimental period (Fig. 2B), even though fluridone treatment resulted in a final absolute germination of 75–80% in both years.

Seedlings that germinated from fluridone-sprayed seeds were bleached, and most did not survive to produce third leaves. Based on above-ground biomass at 84 days after spraying, fluridone was almost 100% effective as a herbicide on *L. rigidum* growing on river sand or sandy loam, but only 70% effective on potting mix (Fig. 2C). Of the crop seeds sown on sandy loam in 2013, none of the seedlings of wheat, canola, bean or chickpea survived the fluridone treatment, while all lupin and field pea seedlings recovered from early, patchy bleaching of the cotyledons and survived to grow normally (Fig. 2D). Time of sowing (0–21 days after fluridone treatment of the soil) and sowing depth (1 or 5 cm) had no effect on wheat seedlings, with all plants bleaching and dying rapidly after germination.

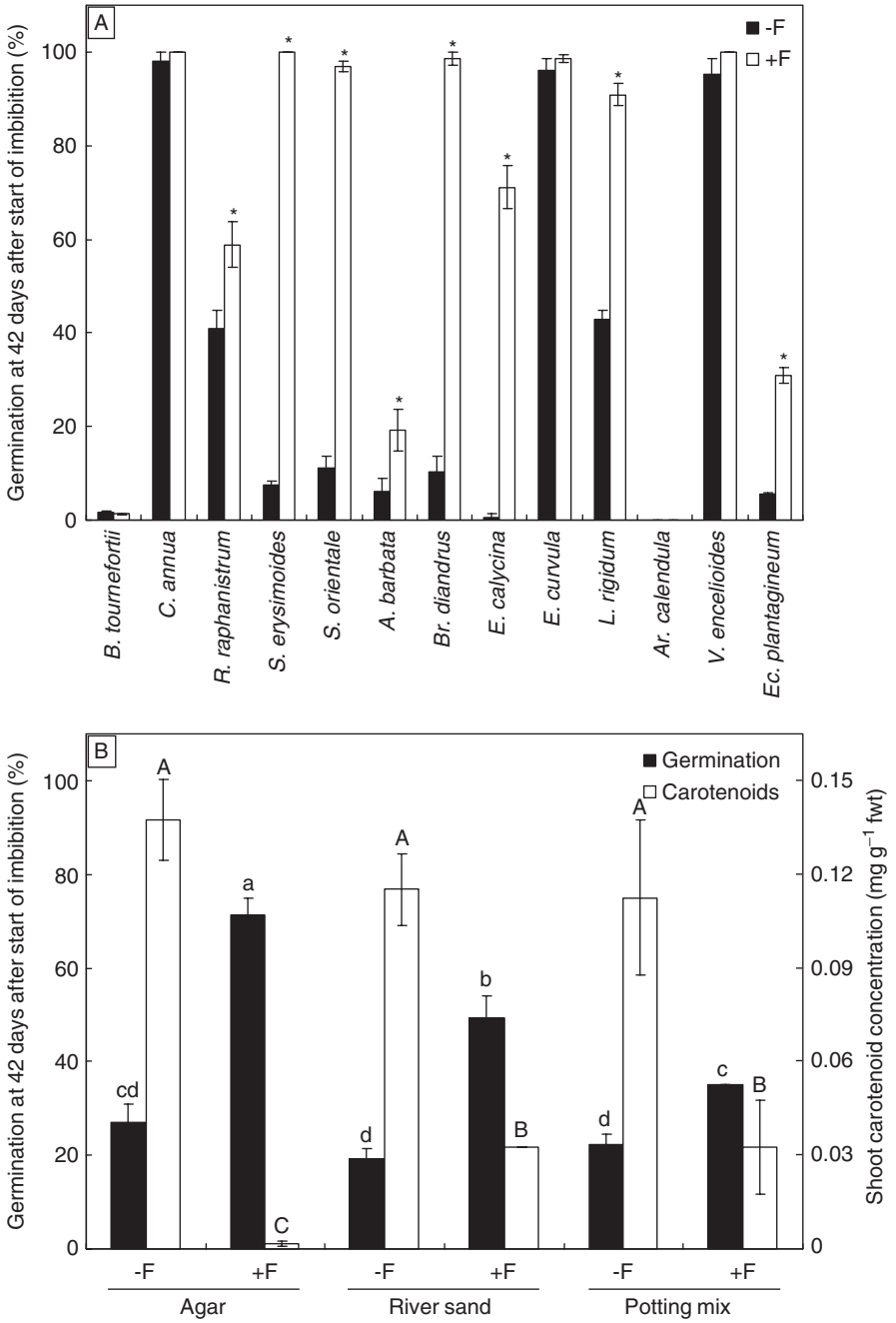

Figure 1. Effect of fluridone under controlled conditions. Seed germination (A, B) and shoot carotenoid concentration in seven-day-old seedlings (B) were measured in response to 50 μM fluridone (F) applied in the germination agar (A) or as a 24 h pulse prior to sowing on fluridone-free media (B). Values are means ± SE ($n = 4$ replicates of 20–50 seeds); asterisks (A) or different letters (B) above columns denote significant differences ($P < 0.05$) between means within each species or parameter.

Owing to a severe lack of rainfall during the experimental period, germination in the field at Quairading was negligible in both the sprayed and unsprayed plots, and thus no conclusions could be drawn from this experiment.

Glasshouse-grown *L. rigidum* plants sprayed with fluridone during flowering yielded seeds that germinated 1.6 times more rapidly than seeds from control plants, but the final germination percentage of the seeds sprayed during development was only slightly higher than that of the controls (Fig. 3). Carotenoid concentrations in the seeds themselves were unaffected by fluridone applied during seed development, but the shoots of the resulting seedlings (at 7 days after the start of imbibition) were partially bleached, containing only around 30% of the carotenoids of seedlings germinated from control seeds (Fig. 3). However, seedlings that were allowed to grow under glasshouse conditions for 60 days completely recovered from their early bleaching and grew normally.

4 DISCUSSION

This study, representing an initial assessment of the potential use of fluridone as a seed germination stimulant plus herbicide for *Lolium*

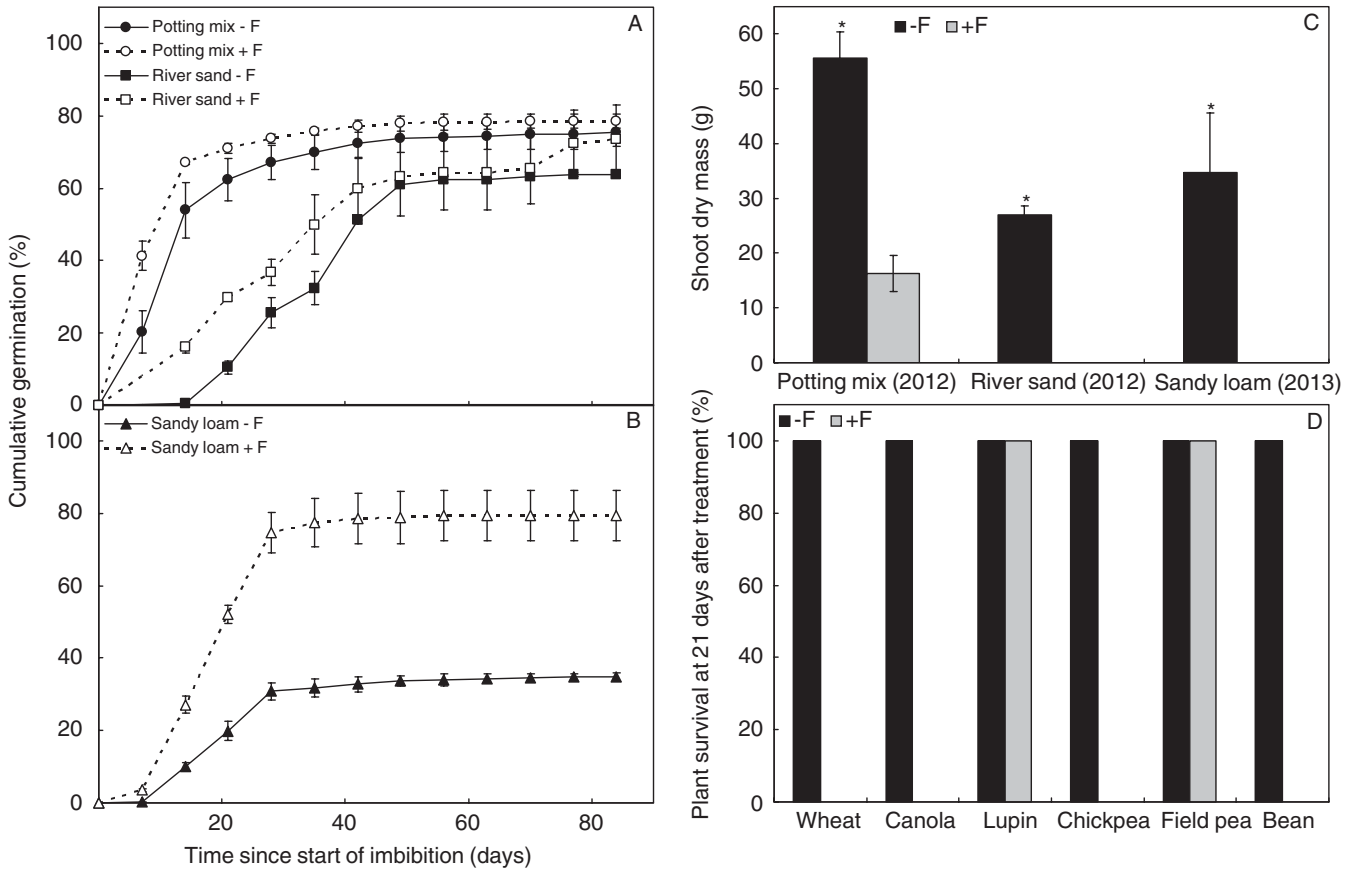

Figure 2. Assessment of fluridone as a combination germination stimulant/herbicide. Seed germination (A, B) and shoot biomass (C) were measured in response to spraying of after-ripened seeds on the surface of potting mix or river sand in 2012 (A) or sandy loam topsoil in 2013 (B) with commercial fluridone (F) at 367 g AI ha^{-1} just after imbibition. Survival of crop plants sown into fluridone-treated sandy loam was also assessed (D); values are means ± SE ($n = 3$ replicates of 300 seeds for A to C and 5–12 seeds for D), and asterisks above columns in (C) denote significant differences ($P < 0.05$) between means within each soil type.

rigidum (and potentially other responsive weed species such as members of *Sisymbrium* and *Bromus*) (Fig. 1A), has demonstrated that fluridone may be a useful tool to reduce the soil weed seed bank in problem fields. The germination of mature seeds sprayed with fluridone on the soil surface was stimulated, and the resulting seedlings were bleached and most were unable to survive. In contrast, application of fluridone during seed development had only small, transient effects on seed germination and seedling bleaching, confirming that, for highest efficacy, fluridone needs to be applied to mature, shed seeds rather than to flowering weed plants.

Fluridone binds to organic matter,[25] which would make it less available in soils with a high organic matter content. The highest herbicidal efficacy of fluridone is obtained in soils with a low organic matter content,[26,27] and these results are reflected in both the seed germination and seedling mortality results of the present study, where after-ripened *L. rigidum* seeds had greater and more prolonged stimulation of germination, and higher seedling mortality, on fluridone-treated river sand (organic carbon 0.8%) compared with potting mix (organic carbon 7.6%). Fluridone was also effective on partially after-ripened seeds sown on sandy loam (organic carbon 2.2%) collected from the field, but its efficacy under authentic field conditions could not be assessed in this preliminary study owing to the lack of rainfall during the experimental period. The majority of Australian cropping soils, and soils in many other parts of the world, have a low organic matter content,[28] such as was measured in the sandy loam from the Western Australian grain belt, and therefore further field tests of fluridone as a germination stimulant plus herbicide in these soils are warranted.

The question of the crop plant-back period in fluridone-treated, low-organic-matter soils is important, as many crops are fluridone susceptible.[29] Early work has shown that fluridone persists in soil for 50–385 days, depending upon the fluridone application rate, soil type and year (Schroeder and Banks[27] and references therein). In Australian cropping, it is a commercial practice to chemically fallow a heavily weed-infested field for a full year in order to reduce the weed burden. Use of fluridone or a similar compound could therefore be very useful in such fields. Iliev *et al.*[30] found that cotton, which is naturally fluridone tolerant, could ameliorate the harmful effects of fluridone on maize, apparently through an (uncharacterised) chemical exuded by cotton plants. It would be interesting to screen other crop and pasture plants for their potential as fluridone safeners, so that a problem field could be remediated more quickly after spraying with fluridone. Pilot experiments in the present study suggest that certain legume crops (lupin and field pea) could be sown soon after a fluridone treatment without sustaining damage.

In conclusion, a chemical such as fluridone applied as a germination stimulant plus herbicide could become a valuable component of an integrated weed management system. This may be particularly useful in southern Australian cropping regions where multiple-herbicide-resistant *L. rigidum* is widespread and

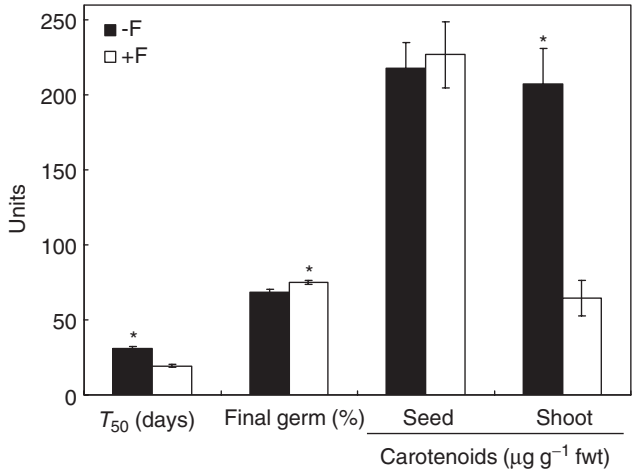

Figure 3. Effect of fluridone applied during seed development. Time to 50% germination (T_{50}, in days), final germination percentage, and carotenoid concentrations (μg g^{-1} fwt) were measured in harvest-ripe seeds produced by *Lolium rigidum* plants sprayed with commercial fluridone (F) at 367 g AI ha^{-1} during flowering; carotenoids were also measured in the shoots of seven-day-old seedlings germinating from these seeds. Values are means ± SE ($n = 20$ replicates of 50 seeds for T_{50} and final germination; $n = 3$ replicates of 50 seed(ling)s for carotenoid concentration); asterisks denote a significant difference ($P < 0.05$) between means within each parameter.

abundant. The only documented case of fluridone resistance so far is in the aquatic weed *Hydrilla*, where dioecious populations exposed to intensive fluridone use in Florida waterways have evolved a mutation in the PDS gene.[16,31] Use of fluridone in a chemical fallow in terrestrial cropping systems would encourage faster germination of the dormant weed seed bank and minimise the need for other herbicides. Modification of the chemical structure of PDS inhibitors such as fluridone could potentially increase their usefulness by minimising problems associated with crop safety and preventing binding of the molecule to soil organic matter.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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