

Cinmethylin controls multiple herbicide-resistant *Lolium rigidum* and its wheat selectivity is P450-based

Roberto Busi,^{a*}  Franck E Dayan,^b  Ian Francis,^c Danica Goggin,^a Jens Lerchl,^d Aimone Porri,^d Stephen B Powles,^a Ci Sun^a and Hugh J Beckie^a 



Abstract

BACKGROUND: Multiple-herbicide resistance in *Lolium rigidum* and other weed species is increasingly exerting pressure on herbicide discovery research for solutions against resistance-prone weeds. In this study we investigate: (i) the responses of *L. rigidum* populations and wheat to the new herbicide cinmethylin in comparison with other pre-emergence herbicides, (ii) the effect of seed burial depths on cinmethylin efficacy and crop selectivity, and (iii) the basis of cinmethylin selectivity in wheat.

RESULTS: Cinmethylin at 400 g ha⁻¹ controls herbicide-susceptible and multiple-resistant *L. rigidum*, with a reduction of >85% in plant emergence and 90% in aboveground biomass. Cinmethylin provides effective control of a large number of field populations of *L. rigidum* with evident resistance to trifluralin. When the wheat seed is buried ≥1 cm below the cinmethylin-treated soil surface, the emergence of crop seedlings is not different from the untreated control. The organophosphate insecticide phorate synergizes cinmethylin toxicity in wheat, with an LD₅₀ of 682 g ha⁻¹ in the absence of phorate versus 109 g ha⁻¹ in the presence of phorate (84% reduction). The synergistic effect of phorate with cinmethylin on herbicide-susceptible *L. rigidum* appears smaller (a 44% reduction in the LD₅₀ of cinmethylin).

CONCLUSIONS: Cinmethylin is effective in controlling multiple-resistant *L. rigidum* and appears safe for wheat when the seed is separated at depth from the herbicide applied to the soil surface. The basis of this metabolism-based selectivity is likely regulated by cytochrome P450 monooxygenases.

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Keywords: cinmethylin; herbicide resistance; *Lolium rigidum*; phorate; selectivity

1 INTRODUCTION

Lolium rigidum (annual ryegrass) infests agricultural areas across the world, particularly in those regions with warm summers and mild winters, for example western USA, South America, southern Europe, the Middle East and parts of Africa (Heap 2019). Across large areas of Australia, *L. rigidum* became a major weed problem when many pastures were converted to wheat or barley crops in the 1970s and infestations of this well-adapted species had to be controlled.¹ This resulted in widespread use of selective pre-emergence herbicides such as the mitosis inhibitor trifluralin, the photosystem (PS)-II inhibitor diuron, and the very long chain fatty acid elongase (VLCFAE) or lipid biosynthesis inhibitors.² Resistance to these herbicides inevitably evolved, but at a relatively slow rate in *L. rigidum* populations exposed to such intense selection pressure.²⁻⁴ The subsequent availability of wheat-selective post-emergence herbicides, such as the acetyl-CoA carboxylase (ACCCase) inhibitor diclofop-methyl and the acetolactate synthase (ALS) inhibitor chlorsulfuron, and the high adoption of no-till cropping systems in the 1990s, further increased the reliance of growers on herbicides, which escalated the selection of herbicide-resistant populations.⁵⁻⁸ The rapid and widespread

occurrence of resistance to selective post-emergence herbicides has been a worldwide phenomenon documented across large areas and different cropping systems.⁹⁻¹¹

Thus, the use of the less resistance-prone soil-applied, pre-emergence herbicides, including trifluralin and the lipid synthesis inhibitor triallate,^{12,13} both introduced in the 1950s, has significantly increased recently in response to widespread resistance to post-emergence herbicides. In the last decade, new wheat-selective uses of other relatively old pre-emergence herbicides

* Correspondence to: R Busi, Australian Herbicide Resistance Initiative, School of Agriculture and Environment, University of Western Australia, Perth, WA 6009, Australia. E-mail: roberto.busi@uwa.edu.au

a Australian Herbicide Resistance Initiative, School of Agriculture and Environment, University of Western Australia, Perth, Australia

b Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO, USA

c BASF Australia Ltd., Tamworth, Australia

d BASF SE, APR/HE-Li475, Limburgerhof, Germany

such as prosulfocarb (also a lipid synthesis inhibitor) as well as newer wheat-selective herbicides such as pyroxasulfone (VLCFAE inhibitor) have been adopted by many Australian farmers. There is the real danger that the evolution of resistance to these herbicides, already increasing along with their usage levels,^{14,15} will result in complex or extensive herbicide cross-resistance patterns.^{16–20}

Before pyroxasulfone was commercialized in 2012, it was investigated in a series of low-dose, recurrent selection studies involving *L. rigidum* to assess its potential for evolution of resistance. Selection with pyroxasulfone resulted not only in resistance to this herbicide, but also imparted cross-resistance to three other lipid synthesis inhibitors: metazachlor, prosulfocarb and triallate.^{18–20} The basis of this resistance is likely metabolism-based, with inhibitor studies indicating glutathione S-transferase²¹ and possibly also cytochrome P450 monooxygenase (P450)²² mediated detoxification of pyroxasulfone. One of the consequences of metabolic herbicide resistance is that the enzyme(s) mediating detoxification of a particular herbicide can potentially also act upon unrelated (and yet-to-be discovered) herbicide chemistries to which the plant has not yet been exposed.^{23,24}

Recently there have been increased efforts and investment by industry on herbicide discovery programmes to find novel molecules with new herbicide modes of action, as well as to develop new uses of existing molecules. For example, cinmethylin was recently reported to inhibit fatty acid thioesterases²⁵ and this herbicide can be selectively used in a number of crops including wheat. In the current study, we tested the efficacy of cinmethylin on populations of *L. rigidum* with different herbicide resistance profiles. In a series of pot experiments, the herbicide was applied to the soil surface pre-emergence, simulating the 'post seeding pre emergence' (PSPE) conditions in the field using two contrasting conditions of crop seed placement (soil surface or buried). The aims of this study were to assess the efficacy of this pre-emergence herbicide on one herbicide-susceptible and three herbicide-resistant *L. rigidum* populations, quantify the level of herbicide selectivity of cinmethylin in spring wheat, and provide insight into the physiological basis of wheat tolerance to cinmethylin.

2 MATERIALS AND METHODS

2.1 Plant material

Four *L. rigidum* populations exhibiting resistance (R) or susceptibility (S) to different herbicide modes of action (Fig. S1; Table S1) were used in the study together with wheat (var. Mace and Magenta). Population S is susceptible to all herbicides and is used extensively as a control in herbicide resistance screening studies.^{5,18,26,27} Population R-AB was originally collected from the Western Australian (WA) wheatbelt in 2003 and characterized as resistant to post-emergence herbicides inhibiting ACCase (group A mode of action) and ALS (group B).⁶ A different pyroxasulfone-susceptible population, also originally collected from the WA wheatbelt in 2003 (i.e. before the commercialization of pyroxasulfone and prosulfocarb), was subjected to recurrent selection under controlled conditions with low doses of pyroxasulfone, resulting in a subpopulation (here referred to as population R-NK₃) with resistance to pyroxasulfone (group K) and prosulfocarb+S-metolachlor (groups N and K₃).²⁰ Population MR is resistant to multiple modes of action, conferred through a combination of target-site mutations and an enhanced capacity for metabolic detoxification of herbicides.² The population as originally collected from the field was resistant to diclofop-methyl,

chlorsulfuron, trifluralin and S-metolachlor, but was susceptible to pyroxasulfone.^{3,28} After recurrent selection with pyroxasulfone as for population R-NK₃, followed by a further two rounds of selection with prosulfocarb+S-metolachlor, the MR population also displayed high levels of resistance to pyroxasulfone, prosulfocarb and triallate.^{18,19}

2.2 Experiment 1: efficacy of the herbicide cinmethylin in the control of *L. rigidum* populations

One thousand seeds (estimated by weight) of each *L. rigidum* population and wheat variety were germinated on 0.6% (w/v) agar-water in a naturally-lit glasshouse at the University of Western Australia between May and September, 2016. At 5 days after the start of imbibition, seedlings with emerged radicles were transplanted into 18-cm diameter plastic pots containing potting mix (50% peat moss, 25% pine bark, 25% washed river sand) and treated with pre-emergence herbicides 2 h after transplantation. Each seed was buried at 1-cm depth (i.e. covered by 1-cm potting mix before herbicide treatment) and the herbicide was applied to the soil surface using a twin-nozzle sprayer calibrated to deliver 120 L water ha⁻¹ at 210 kPa. The herbicide cinmethylin (Luximax, BASF, Melbourne, Australia) was applied at 0, 12, 50, 100, 200, 300, 400 and 600 g ha⁻¹ in a dose-response experiment (recommended label rate in Australia is 375 g cinmethylin ha⁻¹). Following herbicide application, seeds were covered with an additional 0.5 cm of fresh potting mix. For each cinmethylin dose there were four replicate pots containing 26 (*L. rigidum*) or 15 (wheat) seeds. Each pot was an experimental unit. The experiment, which was arranged in a completely randomized design, was repeated and the data pooled for analysis.

2.3 Experiment 2: cross-resistance profiles of *L. rigidum* populations

The full label rate of cinmethylin and three commercial pre-emergence herbicides was applied to 130 field populations of *L. rigidum* collected from approximately 50 different WA farms between December 2018 and March 2019. Sixty seeds of each *L. rigidum* population were placed in 20-cell trays (60 seeds per cell representing one replicate per population) on the soil surface and treated with the following: 3000 g prosulfocarb ha⁻¹ (Arcade, Syngenta, Sydney, Australia); 100 g pyroxasulfone ha⁻¹ (Sakura, Bayer CropScience, Melbourne, Australia), 720 g trifluralin ha⁻¹ (TriflurX, Nufarm, Melbourne, Australia), and 375 g cinmethylin ha⁻¹ (Luximax, BASF). Following herbicide application, seeds were covered with 0.5 cm of potting mix and plant survival assessed for each population as described below. Mean survival values were calculated in response to each of the four pre-emergence herbicides and separated by ANOVA.

2.4 Experiment 3: effect of seed burial depth on response of *L. rigidum* and wheat to cinmethylin

Pre-germinated seeds of *L. rigidum* population S and of wheat were buried in pots (pot size 100 × 100 mm) at depths of 0, 1 or 2.5 cm (i.e. seed not covered, seed covered by 1 cm or 2.5 cm of potting mix) before cinmethylin treatment at three different doses (0, 188 or 400 g ha⁻¹) applied at the soil surface. Following herbicide application, pots were covered with an additional 0.5 cm of potting mix. For each cinmethylin dose and untreated control there were three replicated pots containing 30 seeds for *L. rigidum* and 15 seeds for wheat. The experiment was arranged in a completely randomized design, repeated, and the data were pooled before statistical analysis.

2.5 Experiment 4: basis of cinmethylin selectivity in wheat

An experiment was conducted to compare the response of *L. rigidum* and wheat (var. Mace) to cinmethylin in the presence or absence of the insecticide phorate. Seeds were placed on the soil surface in 20-cell trays and treated with cinmethylin plus or minus phorate. Granular phorate was applied to the soil surface, c. 30 min before the herbicide treatment, at a dose of 0.1 g cell⁻¹ which would correspond to a field dose of 10 kg phorate ha⁻¹.²² Cinmethylin was sprayed on to the soil surface as described above at rates of 0, 12, 25, 50, 100, 150, 200, 250, 300, 400 500 and 600 g ha⁻¹ in a dose–response experiment. Following insecticide and herbicide application, seeds were covered with 0.5 cm of potting mix. For each cinmethylin dose there were five replicated cells containing c. 30 seeds for *L. rigidum* and wheat. The data from the repeated experiments were pooled for analysis.

2.6 Plant assessments

Pots containing wheat and *L. rigidum* seeds were kept well-watered at ≈80% field capacity until assessment. Plant survival (expressed as the percentage of survivors amongst the total number of treated seedlings) and aboveground biomass (expressed as a percentage of the biomass of untreated controls) were evaluated at 28 days after treatment.

Plant biomass was assessed by weighing material that had been oven-dried for 1 week at 60 °C.

2.7 Statistical analysis

The dose–response data to assess the efficacy of cinmethylin on herbicide-susceptible and -resistant *L. rigidum* compared to wheat (Experiment 1) and the experiment examining the physiological basis of cinmethylin selectivity/tolerance in wheat (Experiment 4) were analyzed by nonlinear regression. The DRC package of the statistical software R v.3.4.2 (R Core Team 2017), was used to calculate the herbicide dose causing 50% plant mortality (LD₅₀) or growth reduction (GR₅₀) and estimation of regression coefficients of a three-parameter log-logistic model:

$$Y = \frac{d}{1 + \exp[b(\log x - \log e)]}$$

where d is the upper limit (100%), b is the slope of the curve, x is the herbicide dose, and e is the dose producing a 50% reduction in response. Nonlinear regression assumptions were met by assuming a continuous Gaussian distribution of errors. Statistically significant differences in response between *L. rigidum* populations, between weed and crop plants, and interaction between the herbicide cinmethylin and the insecticide phorate were established by statistical differences in estimated LD₅₀ or GR₅₀ values, and those differences were assessed using the EDcomp function in the DRC package. To quantify the crop selectivity of cinmethylin and to estimate the effect of phorate on selectivity, we calculated a crop–weed herbicide selectivity index (C–W HSI), similar to our previous study.²² The index (%) was obtained by dividing the estimated LD₅₀ or GR₅₀ value of the sample of interest (a specific *L. rigidum* population, or wheat treated with phorate) by that of wheat treated with cinmethylin only and expressing the result as a percentage (see Tables 1 and 2 for examples). Plant survival, expressed as percentage of seedling emergence in response to the application of cinmethylin at the recommended label dose in comparison with single doses of commercial herbicides (Experiment 2), or plant survival observed at two doses of

cinmethylin assessed at 0-, 1- or 2.5-cm sowing depths (Experiment 3), was analyzed by ANOVA. ANOVA assumptions were held under square root arcsine transformations of the survival data. Mean values (survival %) were separated by multiple comparisons with the *post hoc* Tukey's honest significant difference (HSD) test. Significantly different mean values are indicated by different letters ($P < 0.05$). Back-transformed data were plotted using PRISM 7.02, 2016 (GraphPad, San Diego, CA, USA).

3 RESULTS

3.1 Experiment 1: efficacy of the herbicide cinmethylin in the control of *L. rigidum* populations

Cinmethylin selectively controlled *L. rigidum* across a range of doses without substantially affecting wheat plant survival at 400 g ha⁻¹ [Figs 1(A), S2]. However, at the same dose there was a 25% reduction of wheat aboveground biomass [Fig. 1(B)]. Variation was observed among the four *L. rigidum* populations in response to cinmethylin. Unexpectedly, the S population was less effectively controlled by cinmethylin than the R populations (Fig. 1). The reduced efficacy of cinmethylin in controlling S was consistently observed across the range of doses tested so that the S population displayed significantly (two- to eight-fold) higher LD₅₀ and GR₅₀ values than the three R populations (Table 1). Overall, cinmethylin was safe to wheat, as quantified by the crop/weed selectivity index (Table 1). Thus, the greater cinmethylin toxicity towards *L. rigidum* compared to wheat allows its selective control at doses ≥400 g ha⁻¹ (Fig. 1).

3.2 Experiment 2: cross-resistance profiles of *L. rigidum* field populations

The concomitant response of 130 *L. rigidum* field populations to the full recommended dose of cinmethylin was compared to three pre-emergence herbicides highly adopted in Australia, that is prosulfocarb, pyroxasulfone and trifluralin. *Lolium rigidum* populations were effectively controlled by cinmethylin, prosulfocarb and pyroxasulfone with a kill rate ranging from 95% to 98% (Fig. 2). No significant difference was observed among the mean survival of the *L. rigidum* field populations to these three herbicides. Conversely, trifluralin was significantly less effective in controlling *L. rigidum* populations (Fig. 2). As expected, the range of plant survival in response to trifluralin was the largest among the four herbicides tested because of the high levels of resistance found in some of the *L. rigidum* populations (Fig. 2). Approximately 50% of the populations analyzed exhibited some level of trifluralin resistance (survival ≥6%) in response to a trifluralin dosage causing 100% mortality in the S control (data not shown). The median of survival to trifluralin was 6%. Conversely, the median of survival to cinmethylin and pyroxasulfone was 0% due to a large proportion of the field populations being fully susceptible to these two herbicides (Fig. 2). Survival of wheat was not affected by any of the herbicides used (Fig. S3).

3.3 Experiment 3: effect of seed burial depth on response of *L. rigidum* and wheat to cinmethylin

There was ≥90% seedling emergence observed at all burial depths for both wheat and *L. rigidum* (population S) plants in untreated control pots (Fig. 3). Selective control of *L. rigidum* was evident at the two different doses of cinmethylin tested in this study (188 and 400 g ha⁻¹), as emergence of wheat was significantly greater than that of *L. rigidum* (Fig. 3). *Lolium rigidum* mortality slightly decreased when the seed was placed at depth

Table 1. Estimated LD₅₀ and GR₅₀ values (\pm SE) as g cinmethylin ha⁻¹ for four populations of *Lolium rigidum* (LR), treated with various doses of the experimental herbicide cinmethylin, using nonlinear regression analysis to fit herbicide dose versus plant survival and biomass response data. Wheat (var. Magenta) was included as a cinmethylin-resistant control. The crop–weed herbicide selectivity index (C-W HSI) is the ratio of the weed versus the control wheat LD₅₀ or GR₅₀ values, expressed as a percentage (i.e. wheat = 100%). Probability values (*P*) of the difference of estimated LD₅₀ and GR₅₀ values between wheat and *L. rigidum* populations are reported following a *t*-test performed in the DRC package in the software R

	Survival			Biomass		
	LD ₅₀ (SE)	C-W HSI (%)	<i>P</i>	GR ₅₀ (SE)	C-W HSI (%)	<i>P</i>
Wheat	>400	100	—	>400	100	—
S	165 \pm 45	<41	< 0.01	130 \pm 37	<33	< 0.01
R-AB	75 \pm 15	<19	< 0.01	42 \pm 18	<11	< 0.01
R-NK ₃	20 \pm 6	<5	< 0.01	17 \pm 7	<4	< 0.01
MR	70 \pm 16	<18	< 0.01	37 \pm 11	<9	< 0.01
Pooled LR	61 \pm 9	<15	< 0.01	47 \pm 9	<12	< 0.01

Table 2. Estimated LD₅₀ and GR₅₀ values (\pm SE) from cinmethylin dose–response studies assessing plant emergence (%) and growth of wheat (var. Mace) and herbicide-susceptible *L. rigidum* (S-LR) in the absence [– phorate] or presence [+ phorate] of the organophosphate insecticide phorate using nonlinear regression analysis. The crop–weed herbicide selectivity index (C-W HSI) is expressed as the ratio of the weed or wheat versus wheat (no phorate) LD₅₀ or GR₅₀ values, expressed as a percentage. Probability values (*P*) of the difference between estimated LD₅₀ and GR₅₀ values in the absence (–) or presence (+) of phorate were assessed by a *t*-test performed in the DRC package of the software R

	Survival			Biomass		
	LD ₅₀ (SE)	C-W HSI (%)	<i>P</i>	GR ₅₀ (SE)	C-W HSI (%)	<i>P</i>
Wheat - phorate	682 \pm 171	100	—	278 \pm 31	100	—
Wheat + phorate	109 \pm 11	16	< 0.01	67 \pm 8	24	< 0.01
S-LR - phorate	55 \pm 4	8	—	35 \pm 3	13	—
S-LR + phorate	31 \pm 5	5	< 0.01	25 \pm 3	9	N.S.

[Fig. 3(B,C)]. When the wheat seed was placed on the soil surface (0 cm depth), it appeared that var. Mace plants were significantly more sensitive than Magenta to the action of cinmethylin, as emergence decreased by 75% in Mace versus 37% in Magenta at the maximum tested dose of 400 g cinmethylin ha⁻¹ [Fig. 3(A)].

3.4 Experiment 4: basis of cinmethylin selectivity in wheat

We conducted preliminary research to understand the physiological basis of cinmethylin selectivity in wheat, by contrasting the response of wheat and *L. rigidum* plants to cinmethylin in the presence or absence of the organophosphate insecticide phorate, which is a known inhibitor of P450.¹⁹ In the presence of phorate, cinmethylin was toxic to wheat [Fig. 4(A)]. Survival and growth of wheat seedlings in response to a range of cinmethylin doses in the presence of phorate was significantly lower than when the inhibitor was absent (Table 2; Fig. S4). The presence of phorate reduced the selectivity of cinmethylin by 84% (survival) and 76% (growth), as indicated by the reduction in C-W HSI values (Table 2). Such an increased toxicity of cinmethylin to wheat in the presence of phorate may be due to the reduced capacity of wheat plants to detoxify cinmethylin via P450 activity. Conversely, the relative increase in cinmethylin toxicity in the presence of phorate was less dramatic in herbicide-susceptible *L. rigidum* [Fig. 4(B), Table 2]. There was no significant difference in GR₅₀ values for *L. rigidum* observed in response to cinmethylin in the presence or absence of phorate and only a 45% decrease in LD₅₀ values (Table 2).

4 DISCUSSION

Weed infestations challenge profitable and sustainable food and fibre production.²⁹ The removal of agricultural weeds is often laborious, and requires multiple interventions and integrated strategies.³⁰ Thus far, herbicide remains the most common technology utilized by farmers for weed control³¹ due to its efficiency and simplicity. However, the evolution of herbicide resistance in weed species has been an inevitable and difficult-to-manage consequence of over-reliance on herbicides.³² As weeds have adapted to modern, herbicide-intensive crop cultivation systems, new herbicides have been discovered by the crop protection industry.³³ However, there have not been any new herbicide modes of action successfully commercialized over the last three decades, with the predominance of glyphosate-resistant transgenic crops reducing investment in herbicide discovery.³⁴ Only recently has there been an increased effort to develop novel herbicide molecules and new uses of existing herbicides to control the growing number of herbicide-resistant weed species.³⁵ One promising molecule, cinmethylin, is a relatively old herbicide whose mechanism of action was elucidated only recently. Importantly, cinmethylin also has sufficient selectivity towards wheat and barley.²⁵ Cinmethylin inhibits fatty acid thioesterases²⁵ and the current study suggests that its metabolic cross-resistance profile in the *L. rigidum* populations examined is different from the ACCase or VLCFAE inhibitors.^{21,36}

4.1 Selectivity and herbicidal efficacy of cinmethylin

This study aimed to quantify the level of selectivity of cinmethylin in wheat and assess its efficacy against well-characterized

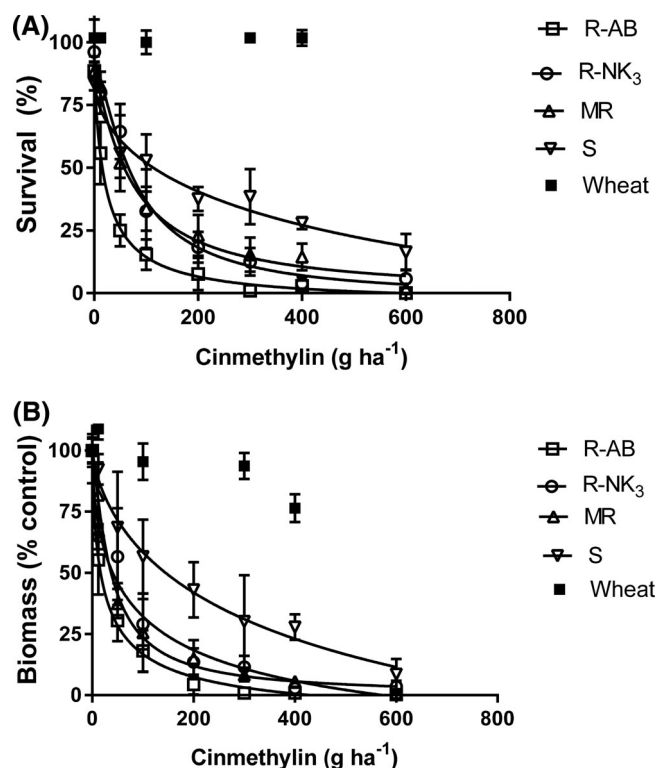


Figure 1. Plant survival (A) and aboveground biomass of survivors (B) assessed at various doses of cinmethylin applied pre-emergence to seeds of wheat (var. Magenta) and four *L. rigidum* populations. Values are means \pm SE ($n = 4$). Lines show the fit of dose–response data to a three-parameter log-logistic model.

multiple herbicide-resistant *L. rigidum* populations. In pot experiments with all seeds at a depth of 1 cm, pre-emergence application of cinmethylin (400 g ha^{-1}) controlled $>85\%$ of *L. rigidum* seedlings in four populations encompassing plants with resistance to multiple modes of action including ACCase- (group A), ALS- (group B), mitosis- (group K_1), lipid- (group N) and VLCFAE- (group K_3) inhibiting herbicides, and was safe to wheat with negligible reductions in plant emergence. A large screening of field populations confirms that cinmethylin effectively controls *L. rigidum*, in that a high proportion of populations appeared fully susceptible to the herbicide. Thus, cinmethylin can be deployed as an additional resource to diversify herbicide use patterns for weed control on farms. When cinmethylin was applied directly to weed and crop seeds on the soil surface, rather than to seeds placed at a depth of ≥ 1 cm, we observed a significantly increased efficacy in weed control but a reduction in crop selectivity due to greater damage being sustained by the wheat (Fig. 3). In our study, seed placement at 1 cm depth could have favoured adsorption of cinmethylin ($K_{oc} = 300 \text{ mL g}^{-1}$) to the organic matter fraction of the potting mix ($\approx 8\%$ organic carbon in the potting mix used). Reduced efficacy of pre-emergence herbicides often is associated with binding of the herbicide to soil organic matter³⁷ and/or crop residue.³⁸

The reduction in the herbicidal efficacy of cinmethylin on buried *L. rigidum* seeds is similar to the situation observed in *Avena fatua* treated with the pre-emergence herbicide pyroxasulfone.³⁹ Several studies have shown the ability of *L. rigidum* to rapidly evolve resistance to wheat-selective herbicides when recurrent selection at a reduced herbicide dose (as received by a buried seed

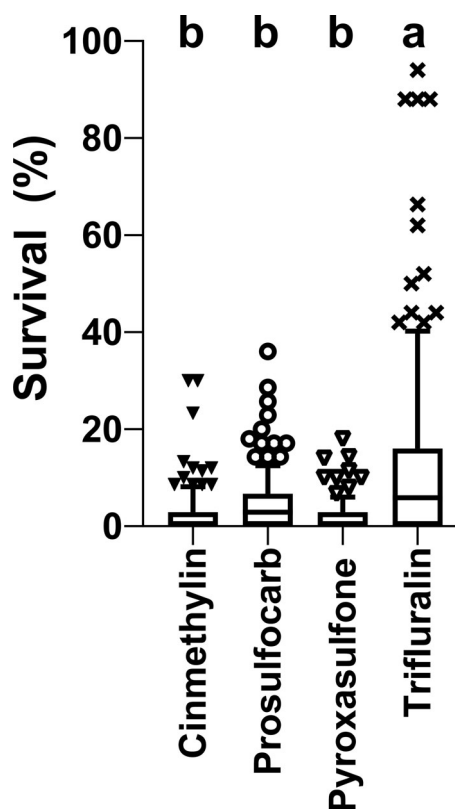


Figure 2. Box and whiskers plot with median line and 10th–90th percentile of plant survival (percentage of plant emergence) observed at the recommended label dose of cinmethylin (375 g ha^{-1}), prosulfocarb (3000 g ha^{-1}), pyroxasulfone (100 g ha^{-1}) and trifluralin (720 g ha^{-1}) applied pre-emergence to seeds of *L. rigidum* field populations collected in 2018/2019 in Western Australia ($n = 130$). Different letters refer to significantly different calculated mean of plant survival values ($P < 0.05$).

compared to a seed lying on the soil surface) acts upon the heritable phenotypic variation of a population.^{40–42} As we observed, there were differences across the four tested *L. rigidum* populations in terms of plant survival in response to cinmethylin, so further research is warranted on the potential of *L. rigidum* to evolve resistance to this herbicide (see also Section 4.2, below). The crop selectivity and efficacy of cinmethylin under field conditions will depend on the relative position of the crop and weed seeds (i.e. sowing depth versus soil disturbance), tillage operations and soil carbon content.

4.2 P450-based cinmethylin selectivity in wheat

In a recent study, we reported that the organophosphate insecticide phorate differentially synergized diverse herbicide modes of action including chlorsulfuron, pyroxasulfone and trifluralin, and allowed the reversal of trifluralin resistance in the MR *L. rigidum* population used in the current study.²² Earlier studies conducted with the same MR population also have established that another organophosphate insecticide, malathion, could inhibit (*in vivo*) the ability of cytochrome P450 to metabolize the ALS-inhibiting herbicide chlorsulfuron.⁴³ The capacity of certain organophosphate insecticides to inhibit the activity of a range of plant P450s responsible for herbicide detoxification is well-documented.^{23,24,44} In this study, we observed reversal of cinmethylin selectivity (i.e. high mortality) in wheat in the presence of phorate

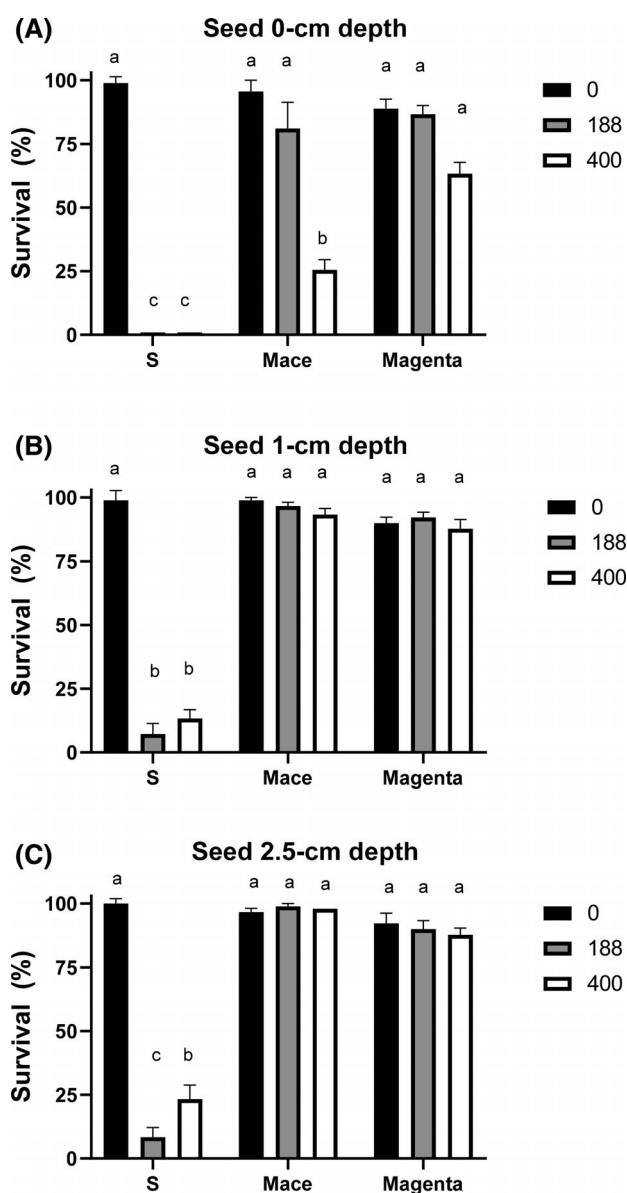


Figure 3. Pooled plant survival (as percentage of plant emergence) assessed at 0, 188 or 400 g cinmethylin ha⁻¹ applied pre-emergence to seeds of wheat (var. Magenta and Mace) and *L. rigidum* population S buried at 0, 1 or 2.5-cm depths. Bars are means ± SE (*n* = 6). Different letters refer to significantly different mean values of plant emergence (percentage) at 0, 188 or 400 g cinmethylin ha⁻¹ for *L. rigidum*, wheat var. Mace or wheat var. Magenta, analyzed all together with multiple comparisons (*P* < 0.05).

(Table 2). Conversely, the toxicity of cinmethylin was only moderately increased by phorate in herbicide-susceptible *L. rigidum*, which, being susceptible to all herbicides, would not be expected to possess enhanced P450 activity. These results suggest that cinmethylin tolerance in wheat is metabolism-based and likely mediated by cytochrome P450 monooxygenase(s). As there is extensive evidence that many herbicide-resistant *L. rigidum* populations resemble wheat in their enhanced ability to metabolize herbicides,^{45–49} this study provides an early speculative insight that *L. rigidum* exposed to intensive cinmethylin selection could evolve resistance trait(s) allowing metabolic detoxification of this herbicide.

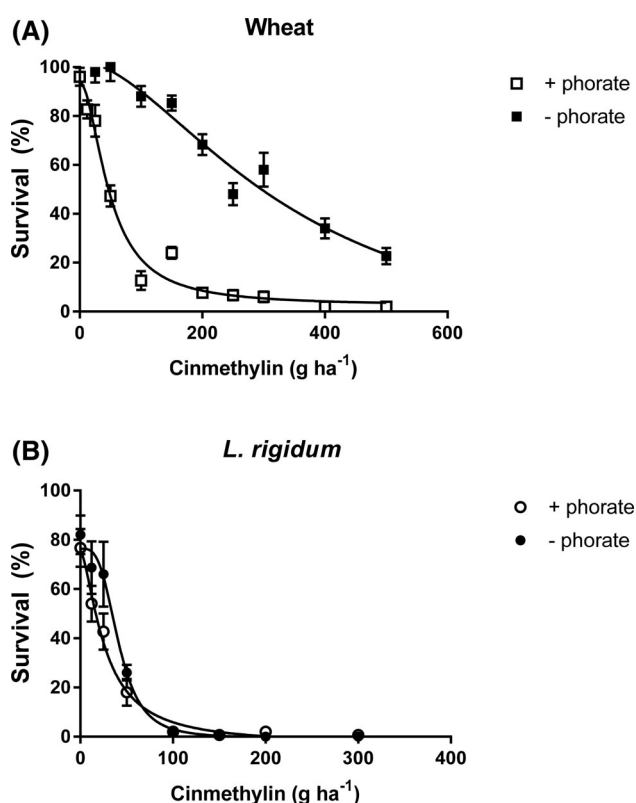


Figure 4. Plant survival (as percentage of plant emergence) of wheat (var. Mace) (A) and herbicide-susceptible (S) *Lolium rigidum* (B) to increasing doses of cinmethylin in the absence [– phorate, solid squares or circles] or presence [+ phorate, open squares or circles] of the organophosphate insecticide phorate. Values are means ± SE (*n* = 10). Lines show the fit of dose-response data to a three-parameter log-logistic model.

4.3 Managing metabolism-based herbicide resistance

An understanding of metabolic resistance to cinmethylin and other pre-emergence herbicides is relevant for improved herbicidal management of multiple-resistant *L. rigidum* populations. We previously have documented that the MR population has constitutive overexpression of two putative resistance-endowing *GST* genes that are associated with phenotypic pyroxasulfone resistance,²¹ as well as enhanced P450-based resistance to chlor-sulfuron and trifluralin.^{22,43} Despite the fact that the MR population displays a very broad profile of cross-resistance to trifluralin,^{2,3} pyroxasulfone, prosulfocarb and triallate,^{18,19} the effective control of population MR by cinmethylin indicates that it does not possess traits that confer resistance to this new herbicide. Similarly, the screening of 130 field-collected populations did not reveal any consistent patterns between the level of resistance to the four herbicides tested (i.e. populations with high trifluralin or prosulfocarb resistance did not necessarily have the highest cinmethylin resistance, and vice versa: Fig. S5), and there was no pattern to the number of populations with resistance to each combination of two or more herbicides (Fig. S6). There are, therefore, no indications that any specific regime of herbicide use should be avoided as strongly promoting the evolution of cross-resistance. The availability and careful use of a diverse array of herbicide modes of action can thus potentially lead to a successful herbicide stewardship programme and long-term effective weed control. A recent modelling study has shown that the capacity of *L. rigidum* to evolve resistance to soil-applied herbicides can be strongly constrained by the adoption of complex

patterns of herbicide rotation and by the use of herbicide mixtures.⁵⁰ The model suggested that the use of herbicide mixtures was particularly important in the initial phases of resistance evolution of small weed populations, when individuals were more likely to be susceptible to the combined action of two different herbicides used as a mixture. Mixtures at high dosages are widely acknowledged as an effective measure to help delay the evolution of quantitative resistance.^{51–53} Thus, diversity in herbicide use and the employment of herbicide mixtures (e.g. trifluralin and pyroxasulfone, cinmethylin and prosulfocarb, or cinmethylin and pyroxasulfone) could decrease the likelihood of selection of multiple resistance traits contributing to herbicide metabolism, and effectively delay the evolution of resistance in *L. rigidum* to soil-applied pre-emergence herbicides.

5 CONCLUSION

Pre-emergence herbicides are the current key tool allowing effective control of multiple-resistant *L. rigidum*, and other major weeds, in the Australian no-till cropping system.^{13,54–56} Trifluralin resistance has been increasing in most regions of the Australian wheatbelt.^{5,56} Before significant levels of field resistance to other wheat-selective herbicides such as prosulfocarb, pyroxasulfone, S-metolachlor and triallate are reported, we suggest here that cinmethylin can provide an effective option to increase diversity in herbicide use to control *L. rigidum*. The most robust use pattern for this new herbicide, in order to minimize the risk of resistance evolution and achieve maximal herbicidal action, would be its use in mixture with another wheat-selective pre-emergence herbicide to complement the chemical properties of each active ingredient (e.g. persistence, solubility, volatility, K_{oc}). Some herbicide rotation patterns in which cinmethylin is included, guided by fundamental knowledge of the likely mechanism(s) of resistance to each herbicide, also could be an effective and robust strategy to delay resistance. Thus, we believe that in the next few years much proactive research is warranted to characterize the risk of resistance evolution to cinmethylin in *L. rigidum*. This would include conducting large-scale field resistance surveys, studies on experimental selection of resistance in controlled environments, and elucidation of the resistance mechanism(s). This research would facilitate the development of sound stewardship guidelines. Ultimately, the combination of proactive research and extension efforts could guide end-users on optimal-use patterns of cinmethylin and other herbicides to extend their longevity and maximize weed control efficacy.

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CONFLICTS OF INTEREST

Authors IF, JL and AP are employees of BASF, which manufactures and sells cinmethylin.

SUPPORTING INFORMATION

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

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