

# Are herbicide mixtures unaffected by resistance? A case study with *Lolium rigidum*

Roberto Busi  | Hugh J. Beckie 

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

## Correspondence

Roberto Busi, Australian Herbicide  
Resistance Initiative, School of Agriculture  
and Environment, University of Western  
Australia, Perth, WA 6009, Australia.  
Email: roberto.busi@uwa.edu.au

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## Abstract

*Lolium rigidum* Gaud., a grass weed species infesting winter field crops, has evolved resistance to the largest number of herbicide modes of action. In this study, 140 field populations of *L. rigidum* were screened with 14 herbicide treatments. Herbicide resistance at the recommended label dosage of pre-emergence (PRE), post-emergence (POST) and binary herbicide mixtures was considered present when plant survival was  $\geq 6\%$ . Plant survival to four acetyl-CoA carboxylase (ACCase) POST herbicides averaged across all populations was approximately 15%, indicating substantial herbicide resistance. In contrast, the mean survival to the PRE treatments was only 2%, reflecting effective control of *L. rigidum*. Herbicide mixtures were the most effective treatments, with a significantly lower resistance frequency than stand-alone herbicides. For example, only 12% of the tested samples were resistant to the mixture of clethodim + butoxydim in comparison with 40% and 61% to either butoxydim or clethodim, respectively. Similarly, 8% of the samples were resistant to the mixture of trifluralin + prosulfocarb versus a much greater frequency of 36% and 51% resistance to prosulfocarb and trifluralin, respectively. Surprisingly, the binary mixtures of trifluralin + triallate or pyroxasulfone + triallate are not affected by resistance (presently) due to the greater efficacy than that of either stand-alone herbicide. Thus, herbicide mixtures can delay the onset of resistance and mitigate the existing levels of herbicide resistance and cross-resistance in *L. rigidum*. Systematic screenings of a large number of field populations could identify the most (cost-) effective herbicide mixtures and foster their informed adoption on farm to mitigate the rapid evolution of weed resistance in lieu of expert assumptions or modelling simulations.

## KEYWORDS

herbicide resistance, IWM, mixtures, selection diversity, weed control

## 1 | INTRODUCTION

Crop pests (weeds, pathogens and insects) have rapidly evolved resistance in response to the intense selection imposed by pesticide use in agriculture (Hawkins *et al.*, 2019). The evolution of resistance to herbicides in weed populations is directly linked to their recurrent exposure to herbicide selection (Neve and Powles, 2005a; Neve and Powles, 2005b). Several empirical studies investigating the underlying

principles of population genetics, ecology and evolution suggest a greater risk of resistance selection when pesticides are alternated (i.e. rotation) instead of used in combination (sequences, mixtures) (Beckie and Reboud, 2009; Lagator *et al.*, 2013a; Lagator *et al.*, 2013b). Simulation modelling has supported the hypothesis of a lower resistance risk with the use of mixtures due to the assumption that the selection of a resistance directly correlates to the initial frequency of resistance alleles in a population (single pesticide use, e.g.  $1 \times 10^{-7}$ )

compared to the multiplicative product of two initial frequencies (use of a mixture, e.g.  $1 \times 10^{-7} \times 1 \times 10^{-7} = 1 \times 10^{-14}$ ) (Gressel and Segel, 1990; Wrubel and Gressel, 1994; Diggle *et al.*, 2003; REX, 2012).

As weeds are one of the greatest biotic constraints to crop yield, the evolution of herbicide resistance traits in competitive weed species is a real threat to sustainable, global food production (Oerke, 2006; Busi *et al.*, 2013; Shaner and Beckie, 2014). The cross-pollinated grass weed *Lolium rigidum* Gaud. (annual ryegrass) is one of the best global example of plants' ability to adapt in response to intense herbicide selection. It is reported that there are *L. rigidum* populations showing resistance to the largest number of herbicide modes of action (MOA), with cases of multiple resistance to seven MOA (Heap, 2020). In Australia, *L. rigidum* is the most damaging weed, and the financial cost of herbicide resistance is estimated to be \$100 million annually (Llewellyn *et al.*, 2016). Since 1982 with the first case worldwide of resistance to acetyl-CoA carboxylase (ACCase)-inhibiting herbicides reported in *L. rigidum* (Heap and Knight, 1982), there has been a steady increase of the frequency of cross-resistance and multiple resistance to acetolactate synthase (ALS) and ACCase-inhibiting herbicides (hereinafter referred to as POST) (Heap and Knight, 1986; Owen *et al.*, 2014). In the last two decades, such a high level of resistance to POST herbicides has resulted in farmers' adoption of pre-emergence (PRE) herbicides that have remained less prone to resistance (Boutsalis *et al.*, 2012; Broster *et al.*, 2012). In the last 20 years in response to widespread resistance to the POST herbicides, farmers have rapidly shifted to soil-applied PRE herbicides for weed control. For example, soil-applied herbicides including trifluralin, triallate, prosulfocarb and pyroxasulfone are widely used for selective control of *L. rigidum* in wheat crops in Australia (Boutsalis *et al.*, 2014; Brunton *et al.*, 2018, 2019, 2020b).

A recent study shows that broad cross-resistance to PRE herbicide is occurring in a few field populations of *L. rigidum* (Brunton

*et al.*, 2018; Brunton *et al.*, 2019; Brunton *et al.*, 2020b). In Australia, there has been increasing adoption of targeted (field-specific) herbicide resistance testing by farmers and consultants, and several geographic random surveys have been conducted in parallel to targeted testing (Boutsalis *et al.*, 2012; Broster *et al.*, 2012; Owen *et al.*, 2014). Despite alarming trends of increasing herbicide resistance incidence in the southern Australian cropping regions due to the proven ability of *L. rigidum* to evolve broad patterns of cross- and multiple resistance to PRE and POST herbicides, there has been insufficient recognition of the role of herbicide mixtures to help mitigate resistance as shown in other systems (Lagator *et al.*, 2013b; Evans *et al.*, 2016) and the impact of additional cost associated with using the full rates of two or more herbicides.

A study was conducted to assess herbicide resistance in *L. rigidum* seed samples collected from 'focus farms' (benchmark farms) in Western Australia that have been practicing harvest weed seed control. We report the efficacy data of a two-year study in which 140 field populations of *L. rigidum* were screened with a series of PRE and POST herbicides. Herbicide resistance was determined by treating plants at the full recommended dosage of PRE and POST herbicides stand-alone in direct comparison with binary mixtures. We report and discuss the observed current levels of resistance to stand-alone versus herbicide mixtures in the context of sustainable crop protection and resistance mitigation.

## 2 | MATERIALS AND METHODS

### 2.1 | Weed sample collection and assay

A total of 140 seed samples of *L. rigidum* was collected from 58 farms at different locations across Western Australia at the end of the growing

**TABLE 1** Herbicide products, formulations, HRAC classification (new from 2020), use (PRE, POST or mixtures) and dosages used to assess plant survival (%) in populations of *Lolium rigidum* (annual ryegrass) collected in Western Australia in 2017–2019 from 'focus' cropped paddocks

Herbicide (formulation)	HRAC Group	Use	Dose active ingredient (g/ha)	Survival % (SE)
Diclofop-methyl (500 g l <sup>-1</sup> )	A (1)	POST	375	39 (2)
Butroxydim (250 g kg <sup>-1</sup> )	A (1)	POST	45	6 (1)
Clethodim (240 g l <sup>-1</sup> )	A (1)	POST	120	12 (1)
Clethodim + Butroxydim	A + A (1 + 1)	POST (mixture)	120 + 45	2 (1)
Prosulfocarb (800 g l <sup>-1</sup> )	N (15)	PRE	2,400	5 (0.5)
Pyroxasulfone (850 g kg <sup>-1</sup> )	K3 (15)	PRE	100	2 (0.3)
Triallate (500 g l <sup>-1</sup> )	N (15)	PRE	1,500	2 (0.3)
Trifluralin (480 g l <sup>-1</sup> )	K1 (3)	PRE	720	12 (2)
Trifluralin + Prosulfocarb	K1 + N (3 + 15)	PRE (mixture)	720 + 2,400	0.9 (0.2)
Trifluralin + Pyroxasulfone	K1 + K3 (3 + 15)	PRE (mixture)	720 + 100	0.8 (0.2)
Trifluralin + Triallate	K1 + N (3 + 15)	PRE (mixture)	720 + 1,500	0.1 (0.0)
Prosulfocarb + Triallate	N + N (15 + 15)	PRE (mixture)	2,400 + 1,500	0.4 (0.1)
Pyroxasulfone + Prosulfocarb	K3 + N (15 + 15)	PRE (mixture)	100 + 2,400	0.2 (0.1)
Pyroxasulfone + Triallate	K3 + N (15 + 15)	PRE (mixture)	100 + 1,500	0.0 (0.0)

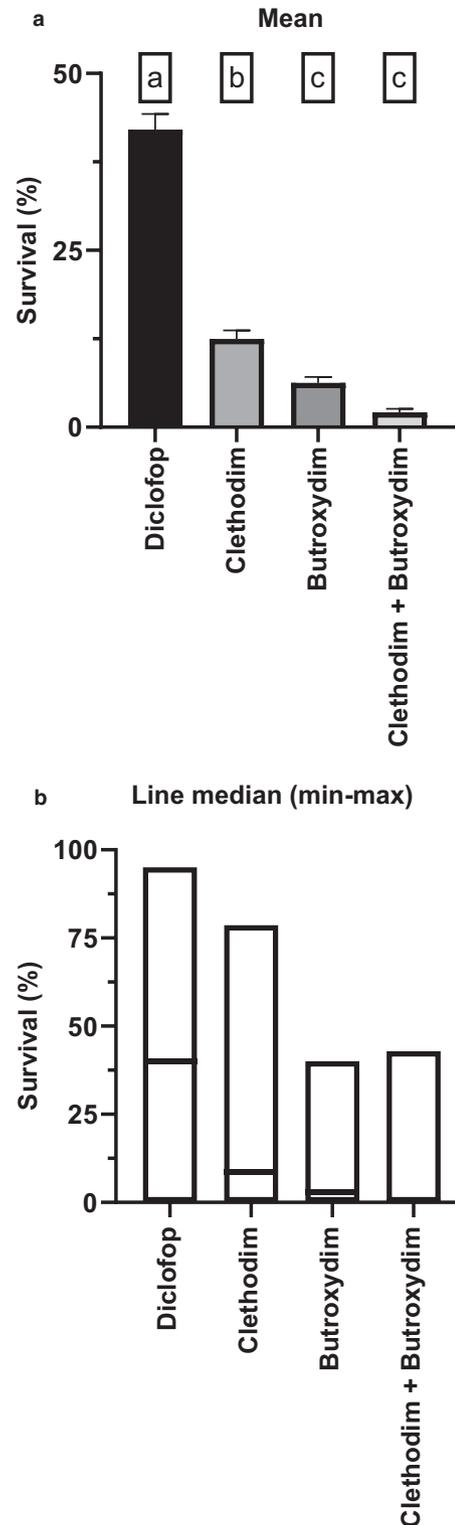
seasons 2017/18 and 2018/19. Fields were chosen according to the grower or consultant's feedback or interests in better understanding the herbicide resistance status of *L. rigidum* infestations. Weed seeds were collected from December to March of each respective season before sowing operations. Samples were collected from a minimum of 20 locations per field and bulked to obtain one population per sampled field. Seeds were cleaned and stored under dry conditions at room temperature for after-ripening before testing for herbicide resistance. Herbicides were applied to the soil (PRE, pre-emergence herbicides) or to two-leaf seedlings at dosages indicated in Table 1 (methodology detailed in Owen *et al.*, 2014). Plant survival was assessed 4 weeks after treatment. Well-characterised herbicide-resistant and herbicide-susceptible weed populations were used as controls. Herbicide resistance was categorised based on specific thresholds of plant survival: survival of 0–5% indicated a herbicide 'susceptible' sample, survival values of 6%–19% identified field populations with a 'developing resistance' status and survival  $\geq 20\%$  was interpreted as herbicide 'resistance'. This weed resistance ranking is generally used because farmers often visually recognise resistance at a level of approximately 20% survival (control escape) in the field. Resistance of  $\geq 20\%$  would result in commercial failure of the herbicide, at which point farmers may stop using the herbicide or consider alternative management options. In contrast, 6%–19% survival indicates that a sufficiently high number of resistant individuals are present in the population that would result in commercial failure the next time the herbicide is applied.

## 2.2 | Data analysis

Plant survival data were expressed as percentages (plants emerged or survived/no. seeds or seedlings treated with herbicides). GraphPad Prism (GraphPad Software, Inc., La Jolla, California, USA) was used to plot the mean percentage of plant survival across all populations tested with each respective PRE and POST herbicide treatment and ANOVA was conducted. The ANOVA assumptions were held under square root arcsine transformations and back-transformed data are presented in all figures. Survival means in response to each herbicide treatment were compared and separated using the post hoc Tukey multiple comparison test ( $\alpha = 0.05$ ). Proportions of resistant, developing resistance and susceptible samples found in response to each herbicide treatment were compared and separated by multiple comparisons with a chi-square heterogeneity test performed using the statistical software *R* with the command *prop.test*. (R Core Team 2019).

## 3 | RESULTS

Averaged over all 140 populations, there was 15% plant survival to the POST herbicides tested, which indicates substantial herbicide resistance in a large proportion of samples. In contrast, the mean survival to PRE herbicides was only 2%, reflecting effective control of the majority of *L. rigidum* field populations tested.



**FIGURE 1** (a) Mean versus (b) range and median plant survival (%) observed across 140 field populations of *Lolium rigidum* collected in Western Australia in 2017–2019 and tested for herbicide resistance at the recommended label dose of three POST ACCase-inhibitor stand-alone herbicides and a binary mixture. Different letters indicate significantly different mean values separated by multiple comparisons by a *post hoc* Tukey test ( $p < 0.05$ ). The ANOVA assumptions were held under square root arcsine transformations

Herbicide POST	Resistance $\geq$ 20% survival	Developing 6–19% survival	Susceptible $\leq$ 5% survival
Diclofop	77 a	17 a	6 a
Clethodim	25 b	37 b	39 b
Butroxydim	10 c	30 b	60 c
Clethodim + Butroxydim	3 d	9 c	88 d

Note: Tested samples were divided into three categories according to the percentage survival observed at the recommended label dose. Herbicide 'Resistance' was diagnosed with  $\geq$  20% survival, 'Developing' resistance with survival ranging between 6% and 19% and 'Susceptible' samples with survival  $\leq$  5%. Within each column, different letters indicate significantly different resistance frequencies (as proportions of samples resistant, developing or susceptible to each respective herbicide). Values were separated by multiple comparisons with a chi-square heterogeneity test performed using the statistical software R with the command *prop.test*.

### 3.1 | Post-emergence herbicides (POST)

The resistance frequency to diclofop-methyl was high, with  $>$ 90% of samples classified as resistant, with a mean plant survival of  $>$ 40% observed in the populations tested (Figure 1). Approximately 60% of the tested samples were clethodim-resistant and the mean survival to clethodim was 12% (Figure 1). The mean survival observed in response to butroxydim was statistically lower than to clethodim, and survival to the mixture of clethodim + butroxydim was the lowest (approx. 2%) but not significantly lower than to butroxydim (Figure 1). Overall frequencies of resistance and developing resistance were significantly lower for clethodim than for diclofop-methyl and lower for butroxydim than clethodim (Table 2). The proportions of samples classified as developing resistance or resistant in response to the mixture clethodim + butroxydim were significantly lower than in response to stand-alone applications of either herbicide (Table 2, Figure S1).

### 3.2 | Pre-emergence herbicides (PRE)

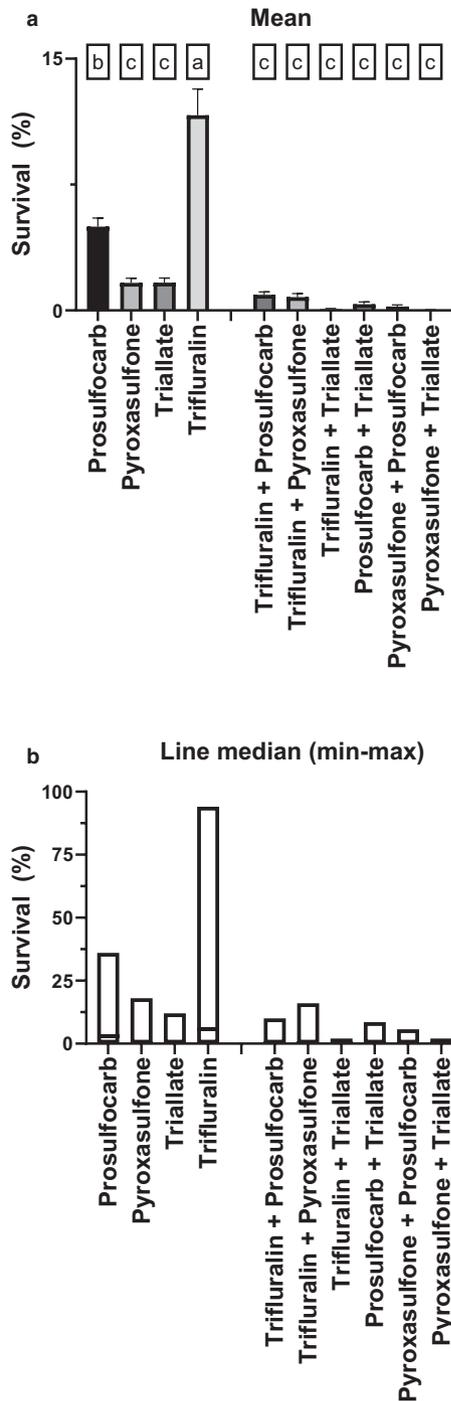
The mean survival to trifluralin (13%) was significantly greater than all other PRE herbicide treatments and ranged from 0% up to approximately 90% in individual populations (Figure 2). Mean survival to prosulfocarb (5%) was significantly lower than trifluralin, but greater than all other PRE herbicides and binary mixtures tested ( $<$ 2%; Figure 2). Trifluralin resistance was found in  $>$ 50% of the samples and prosulfocarb resistance in 36% of samples (Table 3). Highly resistant samples (survival  $>$ 20%) were only found in response to prosulfocarb and to trifluralin and there was a similar frequency of developing resistance to these two herbicides (33–34%), which was significantly higher than all other tested herbicides (Table 3). A significantly lower frequency of resistant samples (8% developing resistance) was found in response to the mixture trifluralin + prosulfocarb, denoting a low proportion of samples with low survival percentages (Figure S2). The frequency of samples 'developing resistance' was similar in all other treatments with the exception of the mixtures trifluralin + triallate and pyroxasulfone + triallate. These two treatments were the most effective, and no tested sample was classified as resistant or developing resistance (Table 3).

**TABLE 2** Herbicide resistance frequencies of 140 populations of *Lolium rigidum* collected in Western Australia in 2017–2019 and tested for herbicide resistance to POST ACCase-inhibiting herbicides and their mixtures

## 4 | DISCUSSION

### 4.1 | Greater efficacy of POST mixtures

Herbicide resistance in *Lolium rigidum* in Australia has been reported to 14 herbicide MOA, the largest number among weed species (Heap, 2020). Repeated herbicide use has selected *L. rigidum* populations with multiple resistance to ALS and ACCase inhibitors that infest the large majority of cropped fields (Broster and Pratley, 2006; Boutsalis *et al.*, 2012; Malone *et al.*, 2014). This study confirms that the frequency of resistance to some ACCase-inhibiting herbicides, including the aryloxyphenoxypropionate herbicide diclofop-methyl and the cyclohexanedione clethodim, in the last decade has remained very high (Boutsalis *et al.*, 2012; Owen *et al.*, 2014). This study indicates a significantly lower frequency of resistance to butroxydim (approx. 40%), in agreement with other studies showing the ability of butroxydim to control clethodim-resistant field populations of *L. rigidum* (Saini *et al.*, 2015a, 2016). The overall survival to the mixture of clethodim + butroxydim, despite tending to be the lowest, was not significantly lower than to butroxydim (Figure 1). Dose-response studies on highly characterised populations of *L. rigidum* confirm a significantly lower LD<sub>50</sub> of butroxydim than clethodim (Busi R, unpublished data). This difference is probably due to the specific characteristics of butroxydim allowing effective binding and subsequent inhibition of the herbicide site of action, despite clethodim resistance-endowing mutations (Jang *et al.*, 2013; Kaundun, 2014). Surprisingly, the frequency of resistance to the mixture of clethodim + butroxydim across a large number of *L. rigidum* populations is significantly reduced to only 12%. A possible explanation of such a sharp drop in resistance frequency in response to this herbicide mixture is the existence of additive interaction between clethodim and butroxydim (Busi R, unpublished data) causing greater herbicide-induced mortality in *L. rigidum* plants exposed to a greater ACCase-inhibitor dosage. It has been consistently observed that ACCase mutations conferring clethodim resistance only result in partial cross-resistance to butroxydim in heterogeneous populations (Saini *et al.*, 2015b) and homozygous lines of *L. rigidum* (Yu *et al.*, 2007). However, despite a large body of literature focused on elucidating the mechanistic



**FIGURE 2** (a) Mean versus (b) range and median plant survival (%) observed across 140 field populations of *Lolium rigidum* collected in Western Australia in 2017–2019 and tested for herbicide resistance at the recommended label dose of four PRE stand-alone herbicides versus six binary mixtures of the same herbicides. Different letters indicate significantly different mean values separated by multiple comparisons by a *post hoc* Tukey test ( $p < 0.05$ )

nature of resistance to individual ACCase-inhibiting herbicides (Délye, 2005; Kaundun, 2014), we note that very few or no studies have investigated the genetic basis of ACCase-inhibitor herbicide resistance in weeds in response to their combined action.

## 4.2 | Greater efficacy of PRE mixtures

In Australia, the rapid increase of resistance to wheat-selective POST herbicides in *L. rigidum* has led to widespread adoption of PRE herbicides such as prosulfocarb, pyroxasulfone, triallate and trifluralin (Busi, 2014). The recent development or introduction of new active ingredients will provide more options for growers (Dayan, 2019; Busi *et al.*, 2020a). Trifluralin has been widely used for almost 60 years to control *L. rigidum* with a historically slow selection and evolution of resistance. However, incidence of resistance is now accelerating to concerning levels based on the results of this study and previous work (Boutsalis *et al.*, 2012). Resistance to thiocarbamate herbicides (e.g. triallate, prosulfocarb) and very long-chain fatty acid elongase (VLCFAE) inhibitors (e.g. pyroxasulfone), initially only reported from experimental evolution studies under controlled conditions (Busi *et al.*, 2012; Busi and Powles, 2013; Busi, 2014; Busi and Powles, 2016), has now occurred in *L. rigidum* field populations with varied cross-resistance profiles (Brunton *et al.*, 2018; Brunton *et al.*, 2019; Brunton *et al.*, 2020b). A recent modelling study indicated that resistance to herbicide mixtures of soil-applied PRE herbicides with distinct MOA would not occur in *L. rigidum* (Busi *et al.*, 2019). Interestingly, the model was calibrated with single-herbicide resistance data obtained in empirical studies (McAlister *et al.*, 1995; Tardif and Powles, 1999; Busi *et al.*, 2012; Busi and Powles, 2013; Busi *et al.*, 2014; Busi and Powles, 2016; Busi *et al.*, 2018), but no study has investigated resistance in response to the application of PRE herbicide mixtures. As the use of herbicide mixtures (pre-formulated and/or 'tank' mixtures) has become an increasingly common practice (i.e. the combination of trifluralin with newer PRE herbicides), there was a clear need to conduct a study to test the actual level of resistance to most commonly adopted two-way herbicide mixtures in comparison with stand-alone PRE herbicides across many field populations of *L. rigidum*. As observed with POST herbicides, there is again clear evidence that mixtures of PRE herbicides provide effective control as the frequency of resistance is low or zero. A very recent survey study, with samples collected across the whole Australian cropping system, has re-confirmed a much lower (up to 10-fold lower) frequency of resistance to herbicide mixtures in *L. rigidum* populations highly resistant to trifluralin (Busi R and Beckie HJ, unpublished). Recent field studies and historical field surveys have consistently indicated that herbicide mixtures and combinations of herbicide MOA are deliberately chosen by farmers to effectively diversify the inherent selection pressure of herbicidal weed control operations (Brunton *et al.*, 2020a; Harries *et al.*, 2020). The current use of PRE herbicides to control *L. rigidum* include at least five MOA, with a few more of recent introduction (e.g. cinmethylin) or under development (e.g. aclonifen, bixlozone, carbetamide, trifludimoxazin) (Busi *et al.*, 2020a; Harries *et al.*, 2020). Recent studies indicate that the mechanistic genetic basis of resistance to PRE herbicides (e.g. lipid, mitosis and VLCFAE inhibitors) is complex and, most likely determined by distinct population-specific traits; there is likely interaction among super families of proteins

Herbicide PRE	Resistance $\geq$ 20%	Developing 6–19%	Susceptible $\leq$ 5%
Prosulfocarb	3 b	33 a	63 b
Pyroxasulfone	0 b	13 b	87 c
Triallate	0 b	11 b	89 c
Trifluralin	17 a	34 a	49 a
Trifluralin + Prosulfocarb	0 b	8 b	92 c
Trifluralin + Pyroxasulfone	0 b	6 b	94 c
Trifluralin + Triallate	0 b	0 c	100 d
Prosulfocarb + Triallate	0 b	3 bc	97 cd
Prosulfocarb + Pyroxasulfone	0 b	3 bc	97 cd
Pyroxasulfone + Triallate	0 b	0 c	100 d

Note: Tested samples were divided into three categories according to the percentage survival observed at the recommended label dose. Herbicide 'Resistance' was diagnosed with  $\geq$  20% survival, 'Developing' resistance with survival ranging between 6% and 19% and 'Susceptible' samples with survival  $\leq$  5%. Within each column, different letters indicate significantly different resistance frequencies (as proportions of samples resistant, developing or susceptible to each respective herbicide). Values were separated by multiple comparisons with a chi-square heterogeneity test performed using the statistical software *R* with the command *prop.test*.

(GSTs, P450s, etc.) involved in non-target-site herbicide detoxification (Yuan *et al.*, 2007; Busi *et al.*, 2014; Yu and Powles, 2014; Brunton *et al.*, 2020b). For example, we have recently documented that resistance to trifluralin can be significantly decreased by recurrent selection with the thiocarbamate prosulfocarb in two *L. rigidum* populations (Busi *et al.*, 2020b). Such an interaction between resistance traits could explain the low frequency of resistance observed to the mixture trifluralin + prosulfocarb or the lack of resistance to trifluralin + triallate, another very similar mixture tested. Thus, there presently are particular herbicide mixtures that are indeed resilient to resistance. Such nil or low levels of resistance are due to multiple factors including a much greater herbicide toxicity delivered by the deployment of the full dose of two herbicides, complex MOA (VLCFAE inhibitors and thiocarbamates) reducing the probability of resistance endowed by a single mutation near the herbicide target enzyme (Busi, 2014; Brunton *et al.*, 2020b; Gaines *et al.*, 2020), functional recessive inheritance of dinitroaniline herbicides (Chen *et al.*, 2019), negative cross-resistance exerted by herbicide use (Busi *et al.*, 2019) or reduced fitness of multiple-resistant populations (Wu *et al.*, 2018; Gaines *et al.*, 2020).

## 5 | SYNTHESIS AND CONCLUSIONS

Farmers demand simplicity in large and highly mechanised crop protection programs to achieve effective control of pests. In some agricultural systems, this has led to a drastic reduction of crop diversity with detrimental consequences to the sustainable deployment of long-term effective pest management strategies (Storkey *et al.*, 2019), including weed control (Storkey and Neve, 2018). By studying the specific case of *L. rigidum*, we have foreseen that

**TABLE 3** Herbicide resistance frequencies of 140 populations of *Lolium rigidum* collected in Western Australia in 2017–2019 and tested for herbicide resistance to PRE herbicides and their mixtures

metabolism-based multiple resistance to soil-applied PRE herbicides has the potential to create major agronomic issues in grain crops. Despite a trend of increasing herbicide resistance incidence in the southern Australian cropping regions due to the formidable ability of *L. rigidum* to evolve broad patterns of cross- and multiple resistance to PRE and POST herbicides, and despite the understanding that herbicide mixtures can deliver greater efficacy in the field, there has been only limited practical recognition of the role of herbicide mixtures to help mitigate herbicide resistance (Norsworthy *et al.*, 2012). In particular, we have observed a remarkable paucity of empirical studies that compare the efficacy and mitigation effects on resistance when herbicides are applied at full dosage as stand-alone versus binary mixtures. This provocative 'insight' contribution in this special issue strongly advocates for herbicide resistance research to systematically monitor the current status of resistance to herbicide mixtures instead of relying on the assumption that cross-resistance between two stand-alone herbicides immediately extends to their combined action. Most studies are restricted to modelling simulations (Jacquemin *et al.*, 2009; Busi *et al.*, 2019), with the risk of predictions not being accurate or plausible as assumptions often refer to target-site resistance only (reviewed by Beckie and Tardif, 2012; Comont *et al.*, 2020).

The data presented here allow us to speculate that it is unlikely that a single generalist phenotype can endow resistance to herbicide mixtures when both components are used at a fully effective dosage. This study provides examples showing that target-site resistance to POST herbicides and non-target-site resistance to PRE herbicides can be overcome by offering direct evidence across many *L. rigidum* populations screened. Evolved resistance to binary herbicide mixtures in individual plants may require the accumulation of multiple traits (at least two genetic traits) conferring resistance to each herbicide component applied at the maximum recommended dosage. Studies involving

large collections of field populations screened with ad hoc herbicide mixtures need to be designed and conducted (Gressel, 2020) to better define the 'developing' patterns of cross and multiple resistance. Effective weed management in modern agricultural systems largely rely on synthetic herbicides and new MOA herbicides are rare (Duke, 2012). Effective herbicide mixtures of 'old' and newly commercialised herbicides need to be identified and results extended to farmers and consultants, thereby providing them with immediate (cost-) effective solutions to mitigate the evolution of herbicide resistance. After a span of over 50 years of increasing incidence and complexity of herbicide resistance in weeds worldwide, the time has finally come for the era of herbicide mixtures to be systematically tested, compared with equally effective stand-alone dosages and deployed to mitigate and manage this phenomenon.

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## CONFLICT OF INTEREST

No conflicts of interest have been declared.

## PEER REVIEW

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## ORCID

Roberto Busi  <https://orcid.org/0000-0002-9022-2111>

Hugh J. Beckie  <https://orcid.org/0000-0002-2659-2265>

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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