Research Article

Received: 18 May 2016 Revised: 10 September 2016 Accepted article published: 19 September 2016 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/ps.4441

Phorate can reverse P450 metabolism-based herbicide resistance in *Lolium rigidum*

Roberto Busi,[a*] Todd Adam Gaines[b] and Stephen Powles[a]

Abstract

BACKGROUND: Organophosphate insecticides can inhibit specific cytochrome P450 enzymes involved in metabolic herbicide resistance mechanisms, leading to synergistic interactions between the insecticide and the herbicide. In this study we report synergistic versus antagonistic interactions between the organophosphate insecticide phorate and five different herbicides observed in a population of multiple herbicide-resistant *Lolium rigidum*.

RESULTS: Phorate synergised with three different herbicide modes of action, enhancing the activity of the ALS inhibitor chlorsulfuron (60% LD_{50} reduction), the VLCFAE inhibitor pyroxasulfone (45% LD_{50} reduction) and the mitosis inhibitor trifluralin (70% LD_{50} reduction). Conversely, phorate antagonised the two thiocarbamate herbicides prosulfocarb and triallate with a 12-fold LD_{50} increase.

CONCLUSION: We report the selective reversal of P450-mediated metabolic multiple resistance to chlorsulfuron and trifluralin in the grass weed *L. rigidum* by synergistic interaction with the insecticide phorate, and discuss the putative mechanistic basis. This research should encourage diversity in herbicide use patterns for weed control as part of a long-term integrated management effort to reduce the risk of selection of metabolism-based multiple herbicide resistance in *L. rigidum*.

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

Keywords: agriculture; herbicide resistance; herbicide solutions; metabolism; ryegrass; weed control

1 INTRODUCTION

Herbicides are an efficient approach to minimising the damaging effect of weeds on crop yield and quality.[1] However, persistent use of herbicides can result in the evolution of herbicide resistance, as reported in many weed species in the last 40 years (reviewed by Heap[2]). Weeds can evolve complex patterns of cross and multiple herbicide resistance, which currently presents a global threat to crop production.[3] As an immediate result, herbicide weed management programmes have become more complicated, including alternation, sequences or mixtures of different herbicide modes of action.[4] Mutations at herbicide target sites and non-target site mechanisms such as enhanced herbicide metabolism can confer cross and multiple herbicide resistance at the individual or population level.[5,6] For example, cytochrome P450 enzymes can unexpectedly facilitate enhanced metabolism (detoxification) of certain herbicide molecules.[7] Cytochrome P450 enzymes capable of herbicide metabolism have been identified in crop species (e.g. maize, rice, wheat) and thus endow crop selectivity (herbicide resistance) to several herbicide modes of action.[8] Similarly, glutathione *S*-transferases (GSTs) facilitate conjugation of some herbicides with the tripeptide glutathione, resulting in metabolic detoxification in crop species.[9] Similarly, evolved herbicide resistance in weed species such as *Echinochloa phyllopogon* or *Lolium rigidum* can be mediated by cytochrome P450 enzymes via enhanced herbicide metabolism.[10–13] More generally, in several grass weed genera, such as *Alopecurus*, *Avena*, *Echinochloa* and *Lolium*, it has been shown that cross-resistance to a broad range of herbicide molecules across modes of herbicide action is endowed by the complementary interaction of enzymatic complexes such as cytochrome P450 monooxygenases, GSTs and glycosyl transferases (GTs).[3,14,15]

Certain organophosphate insecticides have demonstrated inhibitory effects on specific P450 enzymes involved in herbicide detoxification pathways, causing a synergistic interaction between the organophosphate insecticide and the herbicide and leading to greater levels of phytotoxicity in crop plants such as rice,[16] maize[17–19] and wheat[20] and grass weeds such as *E. phyllopogon*[21] or *L. rigidum*.[12,22] Organophosphate insecticides are highly reactive and chemically diverse molecules that possess a phosphorous atom with a covalent bond to either sulphur or oxygen. The competition in P450-mediated reactions between herbicides and insecticides,[12] as well as the oxidative desulphuration of the organophosphate insecticide,[23] can explain the resulting

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

a *Australian Herbicide Resistance Initiative, School of Plant Biology, University of Western Australia, Perth, WA, Australia*

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

enhanced herbicide toxicity. However, there are cases in which the opposite effect is documented and the organophosphate insecticide minimises herbicide phytotoxicity and injury to plants. For example, cotton plants can be protected by the application of the organophosphate insecticides phorate and disulfoton, which inhibit the P450-mediated bioactivation via oxidation of the herbicide clomazone and thus prevent the subsequent production of phytotoxic clomazone metabolites.[24] Similar mechanisms of bioactivation are known for thiocarbamate pre-emergent herbicides (reviewed by Busi[25]). When organophosphate insecticides interact with P450 enzymes that confer herbicide resistance in weeds, the resulting increased toxicity of the mixture of herbicide and insecticide could potentially be exploited to overcome metabolic herbicide resistance. However, to our knowledge, this has not been achieved in practice.

In Australia, selective post-emergence control of *L. rigidum* ryegrass in wheat crops commenced in 1978 with the acetyl Co-A carboxylase (ACCase)-inhibiting herbicide diclofop-methyl, followed in the early 1980s by the acetolactate synthase (ALS)-inhibiting herbicide chlorsulfuron. *Lolium rigidum* (Gaud.) is a genetically diverse, cross-pollinated weed species that is widespread in the southern Australian cropping system and has evolved resistance to many herbicide classes deployed for its control.[5] Heap and Knight[26] reported the first case of cross-resistance to ACCase and ALS herbicides. Such ACCase and ALS cross-resistance in *L. rigidum* is widespread throughout the large southern Australian cropping regions.[27,28] Herbicide bioassays and HPLC analysis have identified major metabolites produced by P450-mediated metabolism of the ALS herbicide chlorsulfuron[12,13] and the ACCase herbicide diclofop,[29] and these studies identified similarities between the resistance mechanisms operating in wheat versus *L. rigidum* plants. Subsequent studies in *L. rigidum* have identified putative overexpressed P450 genes[30] and confirmed the current widespread geographical extent of enhanced metabolic resistance to post-emergence ACCase and ALS herbicides.[31]

The *L. rigidum* population SLR31 (multiple-resistant, hereafter referred to as MR) has been extensively studied because of its complex cross-resistance to the ACCase-inhibitor diclofop-methyl, the ALS inhibitor chlorsulfuron,[26] the microtubule assembly inhibitor trifluralin[32] and the very-long-chain fatty acid elongase (VLCFAE)-inhibiting herbicides S-metolachlor[33] and triallate.[22] Subsequent experimental evolutionary studies with this MR population have selected high-level resistance to the isoxazoline pyroxasulfone[34] and the thiocarbamate prosulfocarb.[35] Thus far, studies have established the non-target-site enhanced herbicide metabolism basis of diclofop-methyl[29] and chlorsulfuron resistance in this MR *L. rigidum* population,[12,13,36] but there is limited evidence of the mechanistic basis of resistance to pre-emergent herbicides such as prosulfocarb, pyroxasulfone, *S*-metolachlor, trifluralin and triallate. These studies report that the broad-spectrum cross-resistance profile observed in the MR population is likely non-target-site-based enhanced metabolism.[22,25] As organophosphate insecticides can mediate the inhibition of P450 enzymes involved in metabolic resistance to specific herbicides, in this study we have investigated the effects of the organophosphate insecticide phorate with five different herbicides belonging to four different chemical classes and with three different modes of action. The objective was to assess whether the organophosphate insecticide phorate can inhibit enhanced metabolism of herbicides, and to investigate the subsequent effects on *L. rigidum* resistant plants. We report and discuss the synergistic effects of phorate on trifluralin, chlorsulfuron and pyroxasulfone versus the antagonistic effects observed with prosulfocarb and triallate in the MR *L. rigidum* population.

2 MATERIALS AND METHODS

2.1 Plant material

The *L. rigidum* MR population has an extensive field history of herbicide selection[33] and exhibits multiple resistance to herbicides across different modes of action, including the ACCase inhibitor diclofop-methyl, the ALS inhibitor chlorsulfuron,[13] the mitosis inhibitor trifluralin[32] and the VLCFAE inhibitor *S*-metolachlor.[33] This MR population was originally susceptible to the new herbicide pyroxasulfone (VLCFAE inhibitor)[34] and to prosulfocarb (VLCFAE inhibitor) and only marginally resistant to triallate.[22] When the MR population was subjected to recurrent selection with below-label doses of pyroxasulfone, resistance to pyroxasulfone and cross-resistance to prosulfocarb and triallate evolved.[35] The *L. rigidum* population (VLR1) is known to be susceptible to all herbicides (hereinafter referred to as 'S control'). Importantly, the herbicide-susceptible S plants did not contain major alleles for herbicide resistance, and VLR1 has been used as a control population in many herbicide resistance studies. Wheat [varieties Cobra (LPB07-0956) or Bonnie Rock (W4901157)] was also included in the dose–response studies to measure herbicide efficacy on crop plants.

2.2 Chlorsulfuron, prosulfocarb, pyroxasulfone, triallate and trifluralin dose–response studies

Plants were grown during the Australian winter season (June–September) in a natural outdoor environment providing normal field growing conditions for *L. rigidum*. The mean temperature recorded was 17.3 °C, and temperatures ranged from 11 °C (minimum) to 27 °C (maximum). Seeds were germinated on 0.6% (w/v) agar medium for 4 days and planted immediately following the eruption of the primordial root at 0.5 cm depth in 17 cm diameter pots containing commercial potting soil (50% peat, 25% sand and 25% pine bark). Twenty-five seeds were transplanted in each pot. After transplanting the seeds, the pots were treated with 0, 5, 15, 45 and 100 g chlorsulfuron ha^{-1} (label rate $1\times = 15$ g ha^{-1}), or with 0, 250, 500, 1000, 2000 and 4000 g prosulfocarb ha^{-1} (label rate $1\times = 2000$ g ha^{-1}), or with 0, 6.25, 12.5, 25, 50, 100, 200 and 400 g pyroxasulfone ha^{-1} (label rate $1\times = 100$ g ha^{-1}), or with 0, 125, 250, 500, 1000, 2000 and 4000 g triallate ha^{-1} (label rate $1\times = 800-1500$ g ha^{-1}) or with 0, 30, 60, 120, 240, 480 and 960 g trifluralin ha^{-1} (label rate $1\times = 480-960$ g ha^{-1}). Just prior to the herbicide treatments (approximately 30 min), the insecticide phorate (Thimet; Barmac Industries, Stapylton, Queensland) was manually applied on the soil surface at a dose of approximately 0.1 g pot^{-1} corresponding to 10 kg phorate AI ha^{-1}. At a dose of 3 kg phorate ha^{-1}, no synergistic activity with chlorsulfuron was observed (data not shown). Thus, all subsequent herbicide dose–response studies were conducted at the one dose of 10 kg ha^{-1}.

There were three replicated pots per herbicide dose, and an individual pot represented an experimental unit. The standard herbicide-susceptible S *L. rigidum* population as well as wheat were used as controls to verify the efficacy of each herbicide dose. Plants were grown in optimal conditions and watered regularly (>80% field capacity). Nitrogen (as NH_4NO_3) was applied (50 mg kg^{-1}) at weekly intervals over the course of the experiment. After 21 days, emerged plants were counted to assess survival, and

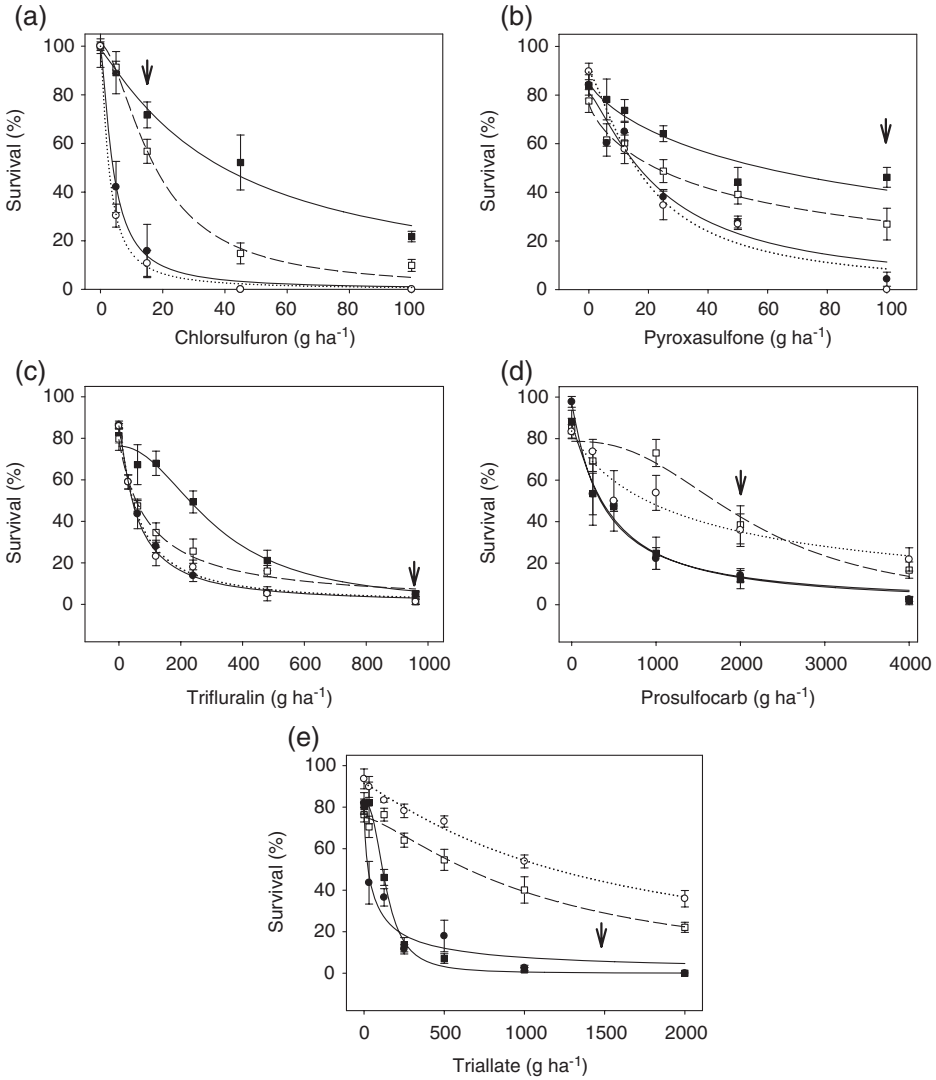

Figure 1. Mean plant survival (emergence) and standard errors in synergistic interaction with (A) chlorsulfuron, (B) pyroxasulfone (in Fig. 1B the solid squares refer to the pyroxasulfone-resistant population MR6 which was subjected to pyroxasulfone recurrent selection as described elsewhere[65]) and (C) trifluralin versus antagonistic interactions with (D) prosulfocarb, and (E) triallate dose–response of the MR *L. rigidum* population. Filled squares and solid lines refer to MR dose–response to each herbicide alone; open squares and a dashed line refer to MR dose–response to each herbicide in combination with phorate; filled circles and a solid line refer to the standard herbicide-susceptible dose–response to each herbicide alone; open circles and a dotted line refer to S dose–response to each herbicide in combination with phorate. Symbols are observed means ± SE ($n = 6$). Arrows indicate mean survival (%) data points obtained at the herbicide recommended field dose. Lines show the fit estimated through non-linear regression analysis with the three-parameter logistic equation to the dose–response data.

above-ground biomass was harvested in each pot. Dry weights of plant biomass were dried at 60 °C for 4 days. Dose–response studies were repeated.

2.3 Statistical analysis

Following appropriate statistical investigation, datasets of the two final dose–response studies, verified not to be significantly different, were pooled prior to analysis. Survival and above-ground biomass data were subjected to non-linear regression analysis as described elsewhere.[35,38] The responses of the MR *L. rigidum* population to different herbicide modes of action in the presence (YES) or in the absence (NO) of the insecticide phorate were measured, and a hypothetical resistance index (RI) or crop–herbicide selectivity index (CSI) of estimated LD_{50} and GR_{50} values was calculated (RI, CSI = ratio between MR population or W/control S population response). Statistical difference was assessed by the SI function in the *drc* package in the software program R v.3.0.2.[39] Graphical data are presented as plant survival (%) for *L. rigidum* or dry weight for wheat (% of control).

3 RESULTS

Phorate alone had no effect on the plants at a dose of 10 kg ha^{-1}. Phorate did not cause mortality or any significant effects on the above-ground plant biomass of the two *L. rigidum* populations or wheat ($P > 0.1$; data not shown). The response of potted *L. rigidum* and wheat plants to each herbicide dose in the presence or absence of phorate is shown in Figs 1 and 2 respectively. As expected, the S *L. rigidum* population was confirmed to be susceptible to chlorsulfuron, prosulfocarb, pyroxasulfone, triallate and trifluralin, with >85% mortality achieved at the recommended label dose of each herbicide (Fig. 1).

Table 1. Pooled data from herbicide dose–response studies (plant survival and growth) to assess resistance or crop safety to chlorsulfuron, prosulfocarb, pyroxasulfone, triallate and trifluralin in *L. rigidum* and wheat (W). Estimated LD_{50} and GR_{50} values expressed as g herbicide ha^{-1} with standard errors in parentheses and resistance index (RI) compared with the standard herbicide-susceptible *L. rigidum* S population (for W, RI indicates a crop–herbicide safety index). Probability values (*P*) of difference between the *L. rigidum* population MR or wheat in response to each herbicide with or without phorate compared with the standard herbicide-susceptible *L. rigidum* S population were assessed by the SI function in the *drc* package in the software program R v.3.0.2 (2013). Parameters *b, d* and *e* of equation ($Y = d / 1 + \exp[b(\log x - \log e)]$) are given for each population tested

Pop.	Herbicide	Phorate	LD_{50}	*b*	*d*	*e*	RI	*P*	GR_{50}	*b*	*d*	*e*	RI	*P*
S	Chlorsulfuron	NO	4 (1)	1.4	100	4	–	–	1.4 (1)	0.76	100	1.4	–	–
S	Chlorsulfuron	YES	2.7 (1)	1.3	100	2.7	0.7	0.45	1.1 (1)	0.94	100	1.1	0.8	0.37
MR	Chlorsulfuron	YES	17 (3)	1.7	101	17	4.3	<0.001	10 (3)	0.88	100	9.7	7	<0.001
MR	Chlorsulfuron	NO	41 (8)	1.1	98	41	10	<0.001	23 (8)	0.81	100	23.2	17	<0.001
W	Chlorsulfuron	YES	98 (4)	7.4	101	98	25	<0.001	98 (10)	2.4	104	97.7	70	<0.001
W	Chlorsulfuron	NO	162 (103)	3.0	101	162	41	<0.001	123 (21)	1.8	109	122.6	88	<0.001
S	Pyroxasulfone	NO	25 (3)	1.3	79	25	–	–	18 (2)	1.7	105	18	–	–
S	Pyroxasulfone	YES	19 (4)	1.4	83	19	0.8	0.16	17 (4)	1.5	98	17	0.9	0.82
MR6[a]	Pyroxasulfone	YES	49 (12)	0.8	77	49	2.0	0.06	52 (13)	1.1	88	52	2.9	0.02
MR6[a]	Pyroxasulfone	NO	91 (23)	0.7	85	91	3.6	0.01	60 (13)	1.0	117	60	3.3	0.008
W	Pyroxasulfone	YES	254 (283)	10	82	254	10	<0.001	383 (353)	2.8	90	377	21	<0.001
W	Pyroxasulfone	NO	290 (1086)	8.7	76	290	12	<0.001	382 (1834)	2.0	93	382	21	<0.001
S	Trifluralin	NO	59 (9)	1.3	87	59	–	–	67 (9)	1.6	107	67	–	–
S	Trifluralin	YES	66 (23)	1.1	85	66	1.1	0.76	79 (14)	2.3	98	79	1.2	0.47
MR	Trifluralin	YES	95 (1)	0.9	80	95	1.5	0.14	62 (21)	0.9	95	62	1.0	0.83
MR	Trifluralin	NO	295 (27)	2.0	80	295	4.8	<0.001	211 (31)	1.3	107	211	2.9	0.001
W	Trifluralin	YES	>960	–	–	–	>16	–	780 (138)	1.5	103	780	12	<0.001
W	Trifluralin	NO	836 (62)	5.5	77	836	14	<0.001	654 (62)	4.0	96	654	9.7	<0.001
S	Prosulfocarb	NO	278 (55)	0.9	91	278	–	–	199 (70)	0.9	104	199	–	–
S	Prosulfocarb	YES	1538 (335)	0.9	83	1538	5.5	<0.001	710 (285)	0.8	98	710	3.5	<0.001
MR	Prosulfocarb	YES	1756 (599)	4.8	77	1756	6.3	0.001	1937 (643)	3.1	90	2171	9.7	0.05
MR	Prosulfocarb	NO	149 (304)	0.9	87	149	0.5	0.67	536 (289)	1.2	99	536	2.6	0.33
MR6[a]	Prosulfocarb	YES	3977 (629)	2.3	84	3977	12	<0.001	3616 (381)	4.2	131	3616	18	0.01
MR6[a]	Prosulfocarb	NO	3772 (450)	3.1	85	3772	11	<0.001	3893 (1076)	1.4	126	3893	19	0.10
W	Prosulfocarb	YES	>4000	–	–	–	>12	–	>4000	–	–	–	>20	–
W	Prosulfocarb	NO	>4000	–	–	–	>12	–	>4000	–	–	–	>20	–
S	Triallate	NO	44 (13)	0.68	92	44	–	–	21 (20)	0.81	100	33	–	–
S	Triallate	YES	1401 (237)	1.1	91	1401	32	<0.001	323 (103)	0.74	99	323	15	<0.001
MR	Triallate	YES	1375 (182)	1.9	74	1375	31	<0.001	345 (186)	0.76	99	345	15	0.44
MR	Triallate	NO	117 (13)	2.5	81	138	2.7	0.36	69 (21)	1.71	97	67	3.2	0.73
W	Triallate	YES	>2000	–	–	–	>45	<0.001	>2000	–	–	–	>61	–
W	Triallate	NO	1965 (209)	1.8	99	1171	44	<0.001	650 (111)	1.3	100	524	16	<0.001

[a] MR6 is pyroxasulfone resistant after being subjected to pyroxasulfone recurrent selection as described elsewhere.[65]

3.1 Phorate synergistic effects

3.1.1 Chlorsulfuron

The chlorsulfuron dose–response study showed more than tenfold resistance in the MR population relative to the S control (Table 1), confirming earlier findings of chlorsulfuron resistance in this population.[12] However, in the presence of phorate, chlorsulfuron was much more effective, with resistance substantially reduced (approximately 60%) (Fig. 1A). The known cytochrome P450 inhibitor malathion was more efficient than phorate in increasing the chlorsulfuron efficacy and reducing the level of chlorsulfuron resistance (approximately 80%; data not shown), as also shown in previous studies.[12] Importantly, in wheat plants, no substantial interactions with phorate were found when plants were treated at normal herbicide doses within the range of the recommended label rate up to 50 g ha^{-1} (supporting information Fig. S1). Estimated LD_{50} and GR_{50} values for wheat were lower in the presence of phorate, yet not significantly different, and they remained more than fivefold greater than the recommended label dose (Tables 1 and 2). Thus, phorate was found to synergise chlorsulfuron in *L. rigidum*, while it was not synergistic in wheat (Fig. 1 and supporting information Fig. S1).

3.1.2 Pyroxasulfone

Pyroxasulfone dose–response studies confirmed pyroxasulfone resistance in the population MR6 (Table 1). Phorate substantially reduced (approximately 45%) the level of pyroxasulfone resistance (Fig. 1B), although plants remained at least twofold resistant relative to the S control population (Table 2). Wheat plant response to pyroxasulfone was not affected by the presence of phorate (Tables 1 and 2, and supporting information Fig. S1).

3.1.3 Trifluralin

The interaction between trifluralin and phorate was similar to chlorsulfuron, with a significant synergistic interaction (Table 1). The trifluralin dose–response analysis confirmed 3–5-fold resistance in the MR population relative to the S control (Table 1).

Table 2. Herbicide effects on *L. rigidum* and wheat plants in the presence and in the absence of the insecticide phorate. Pairwise comparisons were tested to assess synergistic, antagonistic or not significant effects on plant survival and above-ground biomass (A: antagonistic; N/S: not significant; S: synergistic)

Biotype/variety	Herbicide	Phorate	Interactions	P (LD_{50})	P (GR_{50})
SLR31 – MR	Chlorsulfuron	YES	S	0.031	0.193
SLR31 – MR	Chlorsulfuron	NO	–	–	–
Wheat – W	Chlorsulfuron	YES	N/S	0.613	0.312
Wheat – W	Chlorsulfuron	NO	–	–	–
SLR31 – MR6[a]	Pyroxasulfone	YES	N/S	0.191	0.701
SLR31 – MR6[a]	Pyroxasulfone	NO	–	–	–
Wheat – W	Pyroxasulfone	YES	N/S	0.807	0.952
Wheat – W	Pyroxasulfone	NO	–	–	–
SLR31 – MR	Trifluralin	YES	S	0.003	0.050
SLR31 – MR	Trifluralin	NO	–	–	–
Wheat – W	Trifluralin	YES	N/S	0.341	0.343
Wheat – W	Trifluralin	NO	–	–	–
SLR31 – MR	Prosulfocarb	YES	A	<0.001	<0.001
SLR31 – MR	Prosulfocarb	NO	–	–	–
Wheat – W	Prosulfocarb	YES	N/S	0.459	0.902
Wheat – W	Prosulfocarb	NO	–	–	–
SLR31 – MR	Triallate	YES	A	<0.001	<0.001
SLR31 – MR	Triallate	NO	–	–	–
Wheat – W	Triallate	YES	A	<0.001	<0.001
Wheat – W	Triallate	NO	–	–	–

[a] MR6 is pyroxasulfone resistant after being subjected to pyroxasulfone recurrent selection as described elsewhere.[65]

In the presence of phorate, trifluralin resistance was substantially reversed (approximately 70%) to levels not significantly different to the S control population (Fig. 1C). Trifluralin is known to cause some injury to wheat; however, trifluralin injury to wheat was not enhanced by the presence of phorate, which appeared, on the contrary, to deliver some level of protection in wheat plants against this herbicide (supporting information Fig. S1).

3.2 Phorate antagonistic effects

3.2.1 Prosulfocarb

As expected, the MR population was susceptible to the thiocarbamate herbicide prosulfocarb, with estimated LD_{50} and GR_{50} values not significantly different ($P > 0.31$) from the S control population (Table 1 and Fig. 1D). Phorate lowered the efficacy of prosulfocarb (antagonised), resulting in a significant increase in the level of protection against prosulfocarb in *L. rigidum*, with estimated LD_{50} and GR_{50} values up to ninefold greater (Tables 1 and 2). The population MR6, recurrently selected by pyroxasulfone and prosulfocarb, was confirmed as highly resistant to prosulfocarb, but plant survival in this population was not affected by the presence of phorate (Table 1). Wheat plants treated with prosulfocarb were not antagonised by phorate (Table 2 and supporting information Fig. S1).

3.2.2 Triallate

As expected, the results obtained with the thiocarbamate herbicide triallate are very similar to those obtained with the other thiocarbamate herbicide prosulfocarb (Fig. 1E). Without phorate, the MR population was susceptible to triallate and the estimated LD_{50} and GR_{50} values were not significantly different to the standard herbicide-susceptible S population ($P > 0.11$). Conversely, there was a clear reduction in triallate efficacy in the presence of phorate, with much greater survival in MR plants and an estimated LD_{50} value significantly greater than the S control (Table 1). As expected, triallate was significantly less toxic to wheat plants, as they produced much greater above-ground biomass after herbicide treatments than *L. rigidum* plants not exposed to phorate ($P < 0.01$) (Table 1). Phorate did appear to have significant effects on wheat plant survival and biomass exposed to triallate treatments (Table 1). As reported by Patrick and Nalewaja,[40] wheat plants that received phorate treatment had greater survival and produced significantly greater biomass than wheat plants without phorate treatment ($P < 0.001$) (Table 2 and supporting information Fig. S1). The estimated triallate LD_{50} and GR_{50} for wheat plants in the presence of phorate were greater than the highest tested dose (Table 1).

4 DISCUSSION

4.1 Metabolic herbicide resistance can be reversed

This study conducted with a multiple-resistant *L. rigidum* population reports for the first time that the insecticide phorate synergises three different herbicide modes of action. Phorate could reverse resistance to the ALS-inhibiting herbicide chlorsulfuron, the VLCFAE inhibitor pyroxasulfone[41] and the microtubule assembly (mitosis) inhibitor trifluralin.[9] Conversely, phorate antagonised the efficacy of the thiocarbamate herbicides prosulfocarb and triallate in both the herbicide-susceptible and the herbicide-resistant populations.

The capacity of certain organophospate insecticides to inhibit metabolic resistance mechanisms, specifically the metabolic activity of a range of plant cytochrome P450 enzymes responsible for herbicide detoxification via enhanced metabolism, is known.[5,15,42] Our earlier studies conducted with the same MR *L. rigidum* population have established enhanced capacity to metabolise herbicides, mediated by cytochrome P450 enzymes,

as the organophosphate insecticide malathion could inhibit *in vivo* metabolism of the ALS-inhibiting herbicide chlorsulfuron.[12] No such malathion–herbicide interactions were found with prosulfocarb (Busi R *et al.*, unpublished), triallate or trifluralin.[22] Also, in another polygenic multiple-resistant *L. rigidum* population, malathion could not synergise the ACCase-inhibiting diclofop-methyl or the PSII inhibitors simazine or chlortoluron.[43] However, here we found that phorate synergised diverse herbicides, including chlorsulfuron, pyroxasulfone and trifluralin. Thus, this study provides some additional evidence that metabolic herbicide resistance is often polygenic, as different cytochrome P450 gene traits (and possibly others such as GSTs, GTs, etc.) contribute to herbicide metabolism in grass weeds such as *L. rigidum*.[5,44–46]

In *L. rigidum*, different organophosphate insecticides likely interact and/or compete with the binding site of different P450 enzymes involved in metabolic resistance and modulate the inhibition of different metabolic processes involved in detoxification of different herbicides.[43,46,48] The observed strong synergistic interaction between phorate and chlorsulfuron or trifluralin suggests that a gradient of metabolic herbicide resistance reversal can be achieved in *L. rigidum* field populations via the likely inhibition of P450 herbicide-detoxifying enzymes. Thus, large effects, injury of resistant plant phenotypes and herbicide resistance reversal to susceptibility could be expected. However, it is important to emphasise that the broadcast application of a dose equivalent to 10 kg phorate ha^{-1} may not be economically feasible and could raise environmental concerns.

4.2 Knowledge of the mechanism leads to improved management of metabolic herbicide resistance

Similarly to the MR *L. rigidum* population used in this study, across Australia it is common to find *L. rigidum* populations with both target-site and non-target-site resistance traits and thus with multiple resistance to several herbicide modes of action such as ACCase- and ALS-inhibiting herbicides.[31,49–51] A large geographical survey conducted in 2010 across the Western Australian grain belt reported >95% frequency of multiple resistance to ACCase- and ALS-inhibiting herbicides in *L. rigidum*.[28] One response to resistance and failures of post-emergent herbicides has been the adoption of pre-emergent herbicides, especially prosulfocarb, pyroxasulfone and trifluralin.[25] An increasingly common practice has been the use of herbicide mixtures of trifluralin and pyroxasulfone, trifluralin and prosulfocarb or trifluralin and triallate. Our preliminary simulations with the polygenic model PERTH suggest herbicide mixtures can deliver more effective weed control as well as minimise the evolution of resistance in *L. rigidum* (Busi R *et al.*, unpublished).

Several studies have provided some evidence that *L. rigidum* is multiple-resistant owing to enhanced rates of herbicide metabolism, similarly to wheat.[8,52–55] Here, we show significantly greater herbicide toxicity when particular herbicides were used in a mixture with phorate. The explanation for this phenomenon is that chlorsulfuron and trifluralin are likely P450 metabolised in *L. rigidum*, and phorate interferes with this specific metabolic herbicide resistance mechanism. This is somewhat different in wheat plants, where at the tested doses of this study no significant herbicide toxicity or interference between chlorsulfuron and trifluralin with phorate was found. Conversely, phorate did not antagonise prosulfocarb in wheat and in prosulfocarb-resistant *L. rigidum* MR6 plants, and, as shown by McMullan and Nalewaja,[55] in both wheat and *L. rigidum* plants we found similar antagonism between phorate and triallate. This information is valuable, as greater crop safety could be achieved by applying phorate to crop seed (see, for example, Busi *et al.*[56]). Thus, an understanding of metabolic resistance to these herbicides in *L. rigidum* is relevant for an improved herbicide management of multiple-resistant *L. rigidum* populations. The MR population studied here was originally resistant to chlorsufuron,[57] diclofop-methyl and trifluralin,[32,33] and after pyroxasulfone recurrent selection it evolved resistance to pyroxasulfone and cross-resistance to prosulfocarb and triallate.[34,35]

In *L. rigidum*, metabolic resistance to the ALS-inhibiting chlorsulfuron and ACCase-inibiting diclofop-methyl is mediated by more rapid detoxification via a glucose conjugate of hydroxy-chlorsulfuron[58] or the formation of non-toxic ester and aryl-*O*-sugar conjugates respectively.[59] The application of the P450 inhibitor malathion caused inhibition of such a metabolic P450-mediated detoxification mechanism, and it reversed metabolic chlorsulfuron resistance in *L. rigidum* plants.[60] Conversely, malathion[43] or phorate (present study, data not shown) did not synergise metabolic resistance to diclofop-methyl in *L. rigidum*. Whole-plant studies in *L. rigidum* provided indirect evidence for trifluralin metabolism, as cross-resistance to the mitosis inhibitor herbicide pendimethalin in the same population was reversed by synergistic interaction with the P450 inhibitor malathion.[22] There is some information on the biochemical basis of metabolic resistance to the mitosis inhibitor trifluralin[61] or the VLCFAE inhibitor pyroxasuflone in *L. rigidum*.[34] Early studies also report some evidence of trifluralin metabolic biodegradation in soils from bacteria and other mechanisms.[62,63]

In this study we have observed significant phorate synergism with chlorsulfuron and trifluralin, suggesting that P450-mediated herbicide metabolism leads to *in vivo* detoxification of these herbicides in *L. rigidum*. Similarly, the high-level antagonism between prosulfocarb or triallate and phorate confirms that *in vivo* bioactivation of these thiocarbamate *pro*-herbicides is necessary to cause phytotoxic herbicide damage,[64] but also suggests possible metabolism-based resistance mechanism(s) for these two thiocarbamate herbicides. For example, in *Avena fatua*, reduced herbicide metabolic activation via sulfoxidation was found to mediate triallate resistance.[65]

Such a contrasting high-level synergism for chlorsulfuron and trifluralin versus the antagonism of prosulfocarb and triallate with the same insecticide phorate suggests that, among a large number of putative P450 enzymes inhibited by the organophosphate insecticide phorate, common mechanism(s) could mediate herbicide resistance (i.e. herbicide oxidative detoxification) versus increased sensitivity (i.e. *pro*-herbicide oxidative activation) respectively. Our preliminary work shows that, after four generations of pyroxasulfone recurrent selection and two generations of prosulfocarb selection, plants of the population MR6 originally trifluralin resistant have reversed to susceptibility (Busi R and Powles SB, unpublished) (supporting information Fig. S3). In a previous study we have shown monogenic inheritance for trifluralin, prosulfocarb and triallate resistance in the pyroxasulfone-selected MR *L. rigidum* population.[66] Thus, we speculate that a putative cytochrome P450 enzyme(s)/trait could be involved in trifluralin resistance in *L. rigidum* plants, but this same trait could mediate the activation of the two thiocarbamate herbicides, thereby potentially increasing the susceptibility to prosulfocarb and triallate. This hypothesis of negative metabolic cross-resistance between dinitroanilines (e.g. trifluralin) and thiocarbamates (e.g. prosulfocarb and triallate) remains to be tested and confirmed in several *L. rigidum* populations as well as other weed species, but, if confirmed, it could provide insights into management strategies

to control and delay resistance evolution. Additionally, greater P450-mediated oxidative activation of the herbicide clomazone was documented in resistant *E. phyllopogon*, suggesting that other resistance mechanisms, different from P450 detoxification, were involved in resistance.[67]

We have previously hypothesised that, under pyroxasulfone selection, a metabolism-based mechanism similar to wheat could also be present in *L. rigidum* plants. However, as we found partial synergistic interaction between phorate and pyroxasulfone, we suggest (1) that phorate was partially effective in inhibiting putative P450 enzymes mediating pyroxasulfone metabolism or (2) that pyroxasulfone resistance, evolved in MR by low-dose recurrent pyroxasulfone selection, was probably only partially mediated by enhanced cytochrome P450 metabolic activity on pyroxasulfone. The involvement of GSTs mediating herbicide detoxification via glutathione conjugation is well documented for chloroacetamide and thiocarbamate herbicides (reviewed elsewhere[25,64]). One study reported evidence of high levels of GST-mediated pyroxasulfone metabolism in wheat versus low levels in *L. rigidum* plants to explain the herbicide selectivity in crops versus efficacy on weeds respectively.[54]

In conclusion, this study emphasises that diversity in herbicide use, such as using herbicide mixtures (e.g. trifluralin and pyroxasulfone, trifluralin and prosulfocarb or prosulfocarb and pyroxasulfone) or herbicide–insecticide mixtures can increase the efficacy of *L. rigidum* control and can delay resistance evolution in *L. rigidum*. As the insecticide phorate has clear synergistic effects on the herbicide trifluralin in the MR *L. rigidum* population without compromising crop safety, we suggest that this mixture could, at least theoretically, provide an additional measure to control trifluralin-resistant *L. rigidum* populations, especially in geographical areas where trifluralin resistance is more widespread. Phorate acts as a selective synergist in overcoming metabolic resistance in the weed *L. rigidum* without losing the selectivity of the herbicide in wheat, yet further work is warranted to assess the efficacy against field-evolved trifluralin-resistant *L. rigidum* populations and to identify synergistic interactions between trifluralin and phorate at a much reduced dose of the insecticide. In addition, we suggest that phorate could serve as a lead compound in a chemical synthesis programme aimed at producing commercial, crop-selective herbicide synergists that could overcome metabolic herbicide resistance. If crop-selective synergists could be developed, this has potential for a comeback of herbicides that have lost efficacy owing to herbicide resistance evolution. This could provide short-term options to diversify herbicide use patterns for weed control, reduce the risk of resistance and delay the evolution of multiple resistance to residual pre-emergence herbicides that remain effective.

ACKNOWLEDGEMENTS

The Australian Herbicide Resistance Initiative (AHRI) team receives major funding by the Grains Research Development Corporation (GRDC). This research was also partially supported by funding from the Australian Weeds Research Centre (grant AWRC 08–82). Thanks to Jon McCarthy and Craig Geerssen from Barmac Industries for supplying Thimet (phorate). The authors declare no competing financial conflicts of interest.

SUPPORTING INFORMATION

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

REFERENCES

1 Oerke EC, Crop losses to pests. *J Agric Sci* **144**:31–43 (2006).
2 Heap I, Global perspective of herbicide-resistant weeds. *Pest Manag Sci* **70**:1306–1315 (2014).
3 Powles SB and Yu Q, Evolution in action: plants resistant to herbicides. *Annu Rev Plant Biol* **61**:317–347 (2010).
4 Beckie HJ and Tardif FJ, Herbicide cross resistance in weeds. *Crop Prot* **35**:15–28 (2012).
5 Yu Q and Powles S, Metabolism-based herbicide resistance and cross-resistance in crop weeds: a threat to herbicide sustainability and global crop production. *Plant Physiol* **166**:1106–1118 (2014).
6 Délye C, Unravelling the genetic bases of non-target-site-based resistance (NTSR) to herbicides: a major challenge for weed science in the forthcoming decade. *Pest Manag Sci* **69**:176–187 (2012).
7 Zimmerlin A and Durst F, Aryl hydroxylation of the herbicide diclofop by a wheat cytochrome-P-450 monooxygenase – substrate specificity and physiological activity. *Plant Physiol* **100**:874–881 (1992).
8 Werck-Reichhart D, Hehn A and Didierjean L, Cytochromes P450 for engineering herbicide tolerance. *Trends Plant Sci* **5**:116–123 (2000).
9 Cummins I, Wortley DJ, Sabbadin F, He Z, Coxon CR, Straker HE *et al.*, Key role for a glutathione transferase in multiple-herbicide resistance in grass weeds. *Proc Natl Acad Sci USA* **110**:5812–5817 (2013).
10 Yun MS, Yogo Y, Miura R, Yamasue Y and Fischer AJ, Cytochrome P-450 monooxygenase activity in herbicide-resistant and -susceptible late watergrass (*Echinochloa phyllopogon*). *Pestic Biochem Physiol* **83**:107–114 (2005).
11 Iwakami S, Endo M, Saika H, Okuno J, Nakamura N, Yokoyama M *et al.*, Cytochrome P450 CYP81A12 and CYP81A21 are associated with resistance to two acetolactate synthase inhibitors in *Echinochloa phyllopogon*. *Plant Physiol* **165**:618–629 (2014).
12 Christopher J, Preston C and Powles S, Malathion antagonizes metabolism-based chlorsulfuron resistance in *Lolium rigidum*. *Pestic Biochem Physiol* **49**:172–182 (1994).
13 Christopher JT, Powles SB, Liljegren DR and Holtum JAM, Cross resistance to herbicides in annual ryegrass (*Lolium rigidum*). II. Chlorsulfuron resistance involves a wheat-like detoxification system. *Plant Physiol* **95**:1036–1043 (1991).
14 Kreuz K, Tommasini R and Martinoia E, Old enzymes for a new job. Herbicide detoxification in plants. *Plant Physiol* **111**:349–353 (1996).
15 Yuan JS, Tranel PJ and Stewart CN, Non-target-site herbicide resistance: a family business. *Trends Plant Sci* **12**:6–13 (2007).
16 Bowling C and Hudgins H, The effect of insecticides on the selectivity of propanil on rice. *Weeds* **14**:94–95 (1966).
17 Diehl KE, Stoller EW and Barrett M, *In vivo* and *in vitro* inhibition of nicosulfuron metabolism by terbufos metabolites in maize. *Pestic Biochem Physiol* **51**:137–149 (1995).
18 Kreuz K and Fonné-Pfister R, Herbicide–insecticide interaction in maize: malathion inhibits cytochrome P450-dependent primisulfuron metabolism. *Pestic Biochem Physiol* **43**:232–240 (1992).
19 Siminszky B, Corbin FT and Sheldon Y, Nicosulfuron resistance and metabolism in terbufos-treated and naphthalic anhydride-treated corn. *Weed Sci* **43**:163–168 (1995).
20 Zimmerlin A and Durst F, Aryl hydroxylation of the herbicide diclofop by a wheat cytochrome P-450 monooxygenase: substrate specificity and physiological activity. *Plant Physiol* **100**:874–881 (1992).
21 Yasuor H, Osuna MD, Ortiz A, Saldaín NE, Eckert JW and Fischer AJ, Mechanism of resistance to penoxsulam in late watergrass [*Echinochloa phyllopogon* (Stapf) Koss.]. *J Agric Food Chem* **57**:3653–3660 (2009).
22 Tardif FJ and Powles SB, Effect of malathion on resistance to soil-applied herbicides in a population of rigid ryegrass (*Lolium rigidum*). *Weed Sci* **47**:258–261 (1999).
23 Fest C and Schmidt K-J, *The Chemistry of Organophosphorus Pesticides.* JSTOR. Springer-Verlag, Berlin/Heidelberg, Germany (1974).
24 Ferhatoglu Y, Avdiushko S and Barrett M, The basis for the safening of clomazone by phorate insecticide in cotton and inhibitors of cytochrome P450s. *Pestic Biochem Physiol* **81**:59–70 (2005).
25 Busi R, Resistance to herbicides inhibiting the biosynthesis of very-long-chain fatty acids. *Pest Manag Sci* **70**:1378–1384 (2014).
26 Heap I and Knight R, The occurrence of herbicide cross-resistance in a population of annual ryegrass, *Lolium rigidum*, resistant to diclofop-methyl. *Aust J Agric Res* **37**:149–156 (1986).

27 Malone JM, Boutsalis P, Baker J and Preston C, Distribution of herbicide-resistant acetyl-coenzyme A carboxylase alleles in *Lolium rigidum* across grain cropping areas of South Australia. *Weed Res* **54**:78–86 (2014).
28 Owen MJ, Martinez NJ and Powles SB, Multiple herbicide-resistant *Lolium rigidum* (annual ryegrass) now dominates across the Western Australian grain belt. *Weed Res* **54**:314–324 (2014).
29 Yu Q, Han H, Cawthray GR, Wang SF and Powles SB, Enhanced rates of herbicide metabolism in low herbicide-dose selected resistant *Lolium rigidum*. *Plant Cell Environ* **36**:818–827 (2013).
30 Gaines TA, Lorentz L, Figge A, Herrmann J, Maiwald F, Ott MC *et al.*, RNA-Seq transcriptome analysis to identify genes involved in metabolism-based diclofop resistance in *Lolium rigidum*. *Plant J* **78**:865–876 (2014).
31 Han H, Yu Q, Owen MJ, Cawthray GR and Powles SB, Widespread occurrence of both metabolic and target-site herbicide resistance mechanisms in *Lolium rigidum* populations. *Pest Manag Sci* **72**:255–263 (2016).
32 McAlister FM, Holtum JAM and Powles SB, Dinitroaniline herbicide resistance in rigid ryegrass (*Lolium rigidum*). *Weed Sci* **43**:55–62 (1995).
33 Burnet MWM, Barr AR and Powles SB, Chloroacetamide resistance in rigid ryegrass (*Lolium rigidum*). *Weed Sci* **42**:153–157 (1994).
34 Walsh MJ, Fowler TM, Crowe B, Ambe T and Powles SB, The Potential for Pyroxasulfone to Selectively Control Resistant and Susceptible Rigid Ryegrass (*Lolium rigidum*) Biotypes in Australian Grain Crop Production Systems. *Weed Technol* **25**:30–37 (2011).
35 Renton M, Busi R, Neve P, Thornby D and Vila-Aiub M, Herbicide resistance modelling: past, present and future. *Pest Manag Sci* **70**:1394–1404 (2014).
36 Busi R and Powles SB, Cross-resistance to prosulfocarb and triallate in pyroxasulfone-resistant *Lolium rigidum*. *Pest Manag Sci* **69**:1379–1384 (2013).
37 Christopher JT, Powles SB and Holtum JAM, Resistance to acetolactate synthase-inhibiting herbicides in annual ryegrass (*Lolium rigidum*) involves at least two mechanisms. *Plant Physiol* **100**:1909–1913 (1992).
38 Knezevic I, Streibig JC and Ritz C, Utilizing R software package for dose–response studies: the concept and data analysis. *Weed Technol* **21**:840–848 (2007).
39 Ritz C and Streibig JC, Bioassay analysis using R. *J Statist Software* **12**:1–22 (2005).
40 Patrick MM and Nalewaja JD, Triallate antidotes for wheat (*Triticum aestivum*). *Weed Sci* **39**:57–61 (1991).
41 *Production, Supply, and Distribution*. [Online]. Foreign Agricultural Service, USDA, Washington, DC (2016). Available: http://www.fas.usda.gov/psdonline/psdHome.aspx [27 June 2016].
42 Siminszky B, Plant cytochrome P450-mediated herbicide metabolism. *Phytochem Rev* **5**:445–458 (2006).
43 Preston C, Tardif FJ, Christopher JT and Powles SB, Multiple resistance to dissimilar herbicide chemistries in a biotype of *Lolium rigidum* due to enhanced activity of several herbicide degrading enzymes. *Pestic Biochem Physiol* **54**:123–134 (1996).
44 Délye C, Jasieniuk M and Le Corre V, Deciphering the evolution of herbicide resistance in weeds. *Trends Genet* **29**:649–658 (2013).
45 Preston C, Inheritance and linkage of metabolism-based herbicide cross-resistance in rigid ryegrass (*Lolium rigidum*). *Weed Sci* **51**:4–12 (2003).
46 Busi R, Vila-Aiub MM and Powles SB, Genetic control of a cytochrome P450 metabolism-based herbicide resistance mechanism in *Lolium rigidum*. *Heredity* **106**:817–824 (2011).
47 Burnet MWM, Loveys BR, Holtum JAM and Powles SB, Increased detoxification is a mechanism of simazine resistance in *Lolium rigidum*. *Pestic Biochem Physiol* **46**:207–218 (1993).
48 Burnet MWM, Loveys BR, Holtum JAM and Powles SB, A mechanism of chlorotoluron resistance in *Lolium rigidum*. *Planta* **190**:182–189 (1993).
49 Boutsalis P, Gill G and Preston C, Incidence of herbicide resistance in rigid ryegrass (*Lolium rigidum*) across southeastern Australia. *Weed Technol* **26**:391–398 (2012).
50 Malone JM, Boutsalis P, Baker J and Preston C, Distribution of herbicide-resistant acetyl-coenzyme A carboxylase alleles in *Lolium rigidum* across grain cropping areas of South Australia. *Weed Res* **54**:78–86 (2014).
51 Broster JC and Pratley JE, A decade of monitoring herbicide resistance in *Lolium rigidum* in Australia. *Aust J Exp Agric* **46**:1151–1160 (2006).
52 Shimabukuro RH, Walsh WC and Hoerauf RA, Metabolism and selectivity of diclofop-methyl in wild oat and wheat. *J Agric Food Chem* **27**:615–623 (1979).
53 Sweetser PB, Schow GS and Hutchison JM, Metabolism of chlorsulfuron by plants: biological basis for selectivity of a new herbicide for cereals. *Pestic Biochem Physiol* **17**:18–23 (1982).
54 Tanetani Y, Ikeda M, Kaku K, Shimizu T and Matsumoto H, Role of metabolism in the selectivity of a herbicide, pyroxasulfone, between wheat and rigid ryegrass seedlings. *J Pestic Sci* **38**:152–156 (2013).
55 McMullan PM and Nalewaja JD, Triallate antidotes for wheat (*Triticum aestivum*). *Weed Sci* **39**:57–61 (1991).
56 Busi R, Nguyen NK, Chauhan BS, Vidotto F, Tabacchi M and Powles SB, Can herbicide safeners allow selective control of weedy rice infesting rice crops? *Pest Manag Sci* (2016).
57 Heap I and Knight R, The occurrence of herbicide cross-resistance in a population of annual ryegrass, *Lolium rigidum*, resistant to diclofop-methyl. *Aust J Agric Res* **37**:149–156 (1986).
58 Christopher JT, Powles SB, Liljegren DR and Holtum JAM, Cross-resistance to herbicides in annual ryegrass (*Lolium rigidum*) II. Chlorsulfuron resistance involves a wheat-like detoxification system. *Plant Physiol* **95**:1036–1043 (1991).
59 Holtum JAM, Matthews JM, Häusler RE, Liljegren DR and Powles SB, Cross-resistance to herbicides in annual ryegrass (*Lolium rigidum*): III. On the mechanism of resistance to diclofop-methyl. *Plant Physiol* **97**:1026–1034 (1991).
60 Christopher JT, Preston C and Powles SB, Malathion antagonizes metabolism-based chlorsulfuron resistance in *Lolium rigidum*. *Pestic Biochem Physiol* **49**:172–182 (1994).
61 McAlister FM, Holtum JAM and Powles SB, Dinitroaniline herbicide resistance in rigid ryegrass (*Lolium rigidum*). *Weed Sci* **43**:55–62 (1995).
62 Preston C and Malone JM, Inheritance of resistance to 2,4-D and chlorsulfuron in a multiple-resistant population of *Sisymbrium orientale*. *Pest Manag Sci* **71**:1523–1528 (2015).
63 Jugulam M, McLean MD and Hall JC, Inheritance of picloram and 2,4-D resistance in wild mustard (*Brassica kaber*). *Weed Sci* **53**:417–423 (2005).
64 Fuerst EP, Understanding the mode of action of the chloroacetamide and thiocarbamate herbicides. *Weed Technol* **1**:270–277 (1987).
65 Kern AJ, Peterson DM, Miller EK, Colliver CC and Dyer WE, Triallate resistance in *Avena fatua* L. is due to reduced herbicide activation. *Pestic Biochem Physiol* **56**:163–173 (1996).
66 Busi R, Gaines TA, Vila-Aiub MM and Powles SB, Inheritance of evolved resistance to a novel herbicide (pyroxasulfone). *Plant Sci* **217–218**:127–134 (2014).
67 Yasuor H, TenBrook PL, Tjeerdema RS and Fischer AJ, Responses to clomazone and 5-ketoclomazone by *Echinochloa phyllopogon* resistant to multiple herbicides in Californian rice fields. *Pest Manag Sci* **64**:1031–1039 (2008).