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

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**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% LD₅₀ reduction), the VLCFAE inhibitor pyroxasulfone (45% LD₅₀ reduction) and the mitosis inhibitor trifluralin (70% LD₅₀ reduction). Conversely, phorate antagonised the two thiocarbamate herbicides prosulfocarb and triallate with a 12-fold LD₅₀ 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*.

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**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. 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 et al). Weeds can evolve complex patterns of cross and multiple herbicide resistance, which currently presents a global threat to crop production. As an immediate result, herbicide weed management programmes have become more complicated, including alternation, sequences or mixtures of different herbicide modes of action. Mutations at herbicide target sites and non-target site mechanisms such as enhanced herbicide metabolism can confer cross resistance to multiple herbicides at the individual or population level. For example, cytochrome P450 enzymes can unexpectedly facilitate enhanced metabolism of certain herbicide molecules. 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. Similarly, glutathione S-transferases (GSTs) facilitate conjugation of some herbicides with the tripeptide glutathione, resulting in metabolic detoxification in crop species. 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. 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). 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, maize and wheat and grass weeds such as *E. phyllopogon* or *L. rigidum*. 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, as well as the oxidative desulphuration of the organophosphate insecticide, can explain the resulting...
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 thiacarbamate pre-emergent herbicides (reviewed by Busi25). 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 metabolable herbicide resistance. However, to our knowledge, this has not been achieved in practice.

In Australia, selective post-emergence control of L. rigidum rye-grass 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 Knight26 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 chlorsulfuron12,13 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 genes30 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 trifluralin32 and the very-long-chain fatty acid elongase (VLCFAE)-inhibiting herbicides S-metolachlor33 and triallate.22 Subsequent experimental evolutionary studies with this MR population have selected high-level resistance to the isoxazoline herbicide diuron,32 the ALS inhibitor chlorsulfuron,33 and the ACCase inhibitor 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 genes30 and confirmed the current widespread geographical extent of enhanced metabolic resistance to post-emergence ACCase and ALS herbicides.31

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2 MATERIALS AND METHODS

2.1 Plant material

The L. rigidum MR population has an extensive field history of herbicide selection33 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 trifluralin32 and the VLCFAE inhibitor S-metolachlor.33 This MR population was originally susceptible to the new herbicide pyroxasulfone (VLCFAE inhibitor) 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 × 15 g ha−1), or with 0, 250, 500, 1000, 2000 and 4000 g prosulfocarb ha−1 (label rate 1 × 2000 g ha−1), or with 0, 6.25, 12.5, 25, 50, 100, 200 and 400 g pyroxasulfone ha−1 (label rate 1 × 1000 g ha−1), or with 0, 125, 250, 500, 1000, 2000 and 4000 g triallate ha−1 (label rate 1 × 800–1500 g ha−1) or with 0, 30, 60, 120, 240, 480 and 960 g trifluralin ha−1 (label rate 1 × 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 Al 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 NH4NO3) 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
Phorate interaction with pre-em herbicides

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 LD₅₀ and GR₅₀ 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).
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. 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. 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).
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 thio carbamate 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 thio carbamate herbicide triallate are very similar to those obtained with the other thio carbamate 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, 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 and the microtubule assembly (mitosis) inhibitor trifluralin. Conversely, phorate antagonised the efficacy of the thio carbamate herbicides prosulfocarb and triallate in both the herbicide-susceptible and the herbicide-resistant populations.

The capacity of certain organophosphate 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. Our earlier studies conducted with the same MR L. rigidum population have established enhanced capacity to metabolise herbicides, mediated by cytochrome P450 enzymes,

### 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)

<table>
<thead>
<tr>
<th>Biotype/variety</th>
<th>Herbicide</th>
<th>Phorate</th>
<th>Interactions</th>
<th>$P$ (LD$_{50}$)</th>
<th>$P$ (GR$_{50}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLR31 – MR</td>
<td>Chlorsulfuron</td>
<td>YES</td>
<td>S</td>
<td>0.031</td>
<td>0.193</td>
</tr>
<tr>
<td>SLR31 – MR</td>
<td>Chlorsulfuron</td>
<td>NO</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Wheat – W</td>
<td>Chlorsulfuron</td>
<td>YES</td>
<td>N/S</td>
<td>0.613</td>
<td>0.312</td>
</tr>
<tr>
<td>Wheat – W</td>
<td>Chlorsulfuron</td>
<td>NO</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SLR31 – MR6a</td>
<td>Pyroxasulfone</td>
<td>YES</td>
<td>N/S</td>
<td>0.191</td>
<td>0.701</td>
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<td>SLR31 – MR6a</td>
<td>Pyroxasulfone</td>
<td>NO</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Wheat – W</td>
<td>Pyroxasulfone</td>
<td>YES</td>
<td>N/S</td>
<td>0.807</td>
<td>0.952</td>
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<td>Wheat – W</td>
<td>Pyroxasulfone</td>
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<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SLR31 – MR</td>
<td>Trifluralin</td>
<td>YES</td>
<td>S</td>
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<td>0.050</td>
</tr>
<tr>
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<td>Trifluralin</td>
<td>NO</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Wheat – W</td>
<td>Trifluralin</td>
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<td>N/S</td>
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<td>Wheat – W</td>
<td>Trifluralin</td>
<td>NO</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SLR31 – MR</td>
<td>Prosulfocarb</td>
<td>YES</td>
<td>A</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SLR31 – MR</td>
<td>Prosulfocarb</td>
<td>NO</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
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<td>N/S</td>
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<td>0.902</td>
</tr>
<tr>
<td>Wheat – W</td>
<td>Prosulfocarb</td>
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<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SLR31 – MR</td>
<td>Triallate</td>
<td>YES</td>
<td>A</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SLR31 – MR</td>
<td>Triallate</td>
<td>NO</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Wheat – W</td>
<td>Triallate</td>
<td>YES</td>
<td>A</td>
<td>&lt;0.001</td>
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</tr>
<tr>
<td>Wheat – W</td>
<td>Triallate</td>
<td>NO</td>
<td>–</td>
<td>–</td>
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</table>

* MR6 is pyroxasulfone resistant after being subjected to pyroxasulfone recurrent selection as described elsewhere.
as the organophosphate insecticide malathion could inhibit in vitro metabolism of the ALS-inhibiting herbicide chlorsulfuron.\(^2\) No such malathion–herbicide interactions were found with prosulfocarb (Busi et al., unpublished), triallate or trifluralin.\(^2\)

Also, in another polygenic multiple-resistant \(L.\) rigidum population, malathion could not synergise the ACCase-inhibiting diclofop-methyl or the PSII inhibitors simazine or chlorotoluron.\(^4\) 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.\(^5\)

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.\(^6\) 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.\(^7\) A 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.\(^8\) One response to resistance and failures of post-emergent herbicides has been the adoption of pre-emergent herbicides, especially prosulfocarb, pyroxasulfone and trifluralin.\(^9\) 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 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.\(^1\) 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,\(^5\) 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.\(^6\)). 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 chlorsulfuron,\(^7\) diclofop-methyl and trifluralin,\(^8\) and after pyroxasulfone recurrent selection it evolved resistance to pyroxasulfone and cross-resistance to prosulfocarb and triallate.\(^9\)

In \(L.\) rigidum, metabolic resistance to the ALS-inhibiting chlorsulfuron and ACCase-inhibiting diclofop-methyl is mediated by more rapid detoxification via a glucose conjugate of hydroxy-chlorsulfuron\(^8\) or the formation of non-toxic ester and aryl-O-sugar conjugates respectively.\(^9\) 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.\(^10\) Conversely, malathion 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.\(^2\) There is some information on the biochemical basis of metabolic resistance to the mitosis inhibitor trifluralin\(^6\) or the VLCFAE inhibitor pyroxasulfone in \(L.\) rigidum.\(^2\) Early studies also report some evidence of trifluralin metabolic biodegradation in soils from bacteria and other mechanisms.\(^2\)

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,\(^6\) 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.\(^5\)

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.\(^6\) 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 chloroacetamid and thiocarbamate herbicides (reviewed elsewhere21,64). 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