Research Article

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Metribuzin resistance via enhanced metabolism in a multiple herbicide resistant *Lolium rigidum* population

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Abstract

BACKGROUND: The photosystem II (PSII)-inhibiting herbicides are important for Australian farmers to control *Lolium rigidum* Gaud. and other weed species in trazine tolerant (TT)-canola fields. A *L. rigidum* population (R) collected from a TT-canola field from Western Australia showed multiple resistance to PSII, acetyl-coenzyme A carboxylase (ACCase) and acetolactate synthase (ALS) inhibitors. The mechanisms of multiple resistance in this R population were determined.

RESULTS: The R population showed a low-level (about 3.0-fold) resistance to the PSII-inhibiting herbicides metribuzin and atrazine. Sequencing of the *psbA* gene revealed no differences between the R and susceptible (S) sequences. Furthermore, [^{14}C]-metribuzin experiments found no significant difference in metribuzin foliar uptake and translocation between the R and S plants. However, [^{14}C]-metribuzin metabolism in R plants was 2.3-fold greater than in S plants. The cytochrome P450 monooxygenase inhibitor piperonyl butoxide (PBO) enhanced plant mortality response to metribuzin and atrazine in both R and S populations. In addition, multiple resistance to ALS and ACCase inhibitors are due to known resistance mutations in ALS and ACCase genes.

CONCLUSION: The results demonstrate that enhanced metribuzin metabolism likely involving cytochrome P450 monooxygenase contributes to metribuzin resistance in *Lolium rigidum*. This is the first report of metabolic resistance to the PSII-inhibiting herbicide metribuzin in Australian *Lolium rigidum*.

Keywords: metribuzin; metabolic resistance; multiple herbicide-resistant; *Lolium rigidum*

1 INTRODUCTION

The herbicide metribuzin inhibits photosynthesis at photosystem II (PSII) by competing with plastoquinone at the Q_B-binding site on the D1 protein, thus inhibiting electron transport from Q_A to Q_B, resulting in photooxidation and plant death. Metribuzin controls a range of dicot and grass weeds in several crops such as soybeans (*Glycine max* (L.) Merrill), potatoes (*Solanum tuberosum* L.), tomatoes (*Lycopersicon esculentum* Mill.), lupin (*Lupinus angustifolius* L.) and wheat (*Triticium aestivum* (L.) Thell). Over-reliance on herbicides to manage weeds has inevitably resulted in the evolution of herbicide-resistant weed populations. The first case of metribuzin resistance was reported in *Amaranthus powelli* S. Watson in the United States.[1] Since then, globally, biotypes of 74 weed species have been reported to be resistant to PSII-inhibiting herbicides.[2]

Herbicide resistance mechanisms are broadly divided into target-site resistance (TSR) and non-target-site resistance (NTSR). Frequently resistance to PSII-inhibiting herbicides is endowed by the TSR mechanism of chloroplast *psbA* gene mutations causing amino acid substitutions in the D1 protein. So far, eight *psbA* gene mutations including Ser-264-Gly,[3] Ser-264-Thr,[4] Val-219-Ile,[5] Asn-266-Thr,[6] Ala-251-Val,[7] Phe-255-Ile,[8] Leu-218-Val,[9] and Phe-274-Val[10] have been reported in field-evolved, PSII inhibitor- resistant weedy plant species. NTSR involves mechanisms that minimize the amount of active herbicide reaching the target (e.g. reduced herbicide uptake or translocation, increased herbicide sequestration or metabolism). Herbicide resistance due to enhanced herbicide metabolism (metabolic resistance) can endow resistance not only to existing herbicides but also potentially to yet-to-be commercialized herbicides, and is now increasingly recognized as a looming threat to herbicide sustainability and thus crop production.[11]

Lolium rigidum is a damaging weed in global field crops, and especially in Australia.[2] Many *Lolium rigidum* populations have evolved metabolic resistance to herbicides of different modes of action (or chemistries).[12,13] Metribuzin metabolism is known in naturally tolerant crops (e.g. soybean, wheat)[14,15] and field-evolved metribuzin resistant weeds (e.g. *Raphanus raphanistrum* L.).[16] In crops metribuzin metabolism is either via deamination and dethiomethylation to form deaminated metribuzin (DA),

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diketo metribuzin (DK) and deaminated diketo metribuzin (DADK)[14,17,18] or by *N*-glucoside and homoglutathione conjugations of metribuzin.[19,20] Enhanced herbicide metabolism has long been implicated to endow resistance to dissimilar herbicide chemistries including the PSII herbicides chlorotoluron and simazine in multiple resistant *Lolium rigidum* populations.[21,22] Here, working with a multiple resistant *Lolium rigidum* population, we show that resistance to the PSII-inhibiting herbicides in this population is due to non-target-site enhanced herbicide metabolism. We also show that multiple resistance to acetolactate synthase (ALS)- and acetyl-coenzyme A carboxylase (ACCase)-inhibiting herbicides in this population is due to target-site gene mutations.

2 MATERIALS AND METHODS

2.1 Plant materials

The putative metribuzin resistant (R) *Lolium rigidum* population was collected in 2016 in a trazine tolerant (TT)-canola trial from Atlas farms, Calingiri, Western Australia. The susceptible (S) biotype, VLR1, had no history of herbicide application and is susceptible to all herbicides registered for *Lolium rigidum* control.[12,13] This field-collected R without further herbicide selection (unless otherwise specified), together with the standard S populations, were used in this study. The R seeds were stored in a 37 °C room for 2 weeks to break dormancy prior to the experiment, and then kept at room temperature (RT).

2.2 Dose response to herbicides

Seeds of the R and S plants were placed on moist filter paper at 4 °C for 7 days and germinated at room temperature for 4 days. Germinating seeds were transplanted into 18-cm diameter plastic pots containing potting soil (50% peatmoss, 25% sand and 25% pine bark) with 20 seedlings per pot and three replicate pots per treatment. The pots were placed in a glasshouse with average day/night temperatures of 30 °C/22 °C under natural sunlight. When seedlings reached the two- to four-leaf stage, they were treated with metribuzin (Mentor WG, Adama Australia Pty Ltd, St Leonards, NSW, Australia) at 0, 4.69, 9.38, 18.75, 37.5, 75, 150 g ha^{-1} for S and 0, 18.75, 37.5, 75, 150, 300, 600, 1200 g ha^{-1} for R, and atrazine (Gesaprim Granules 900 WG, Syngenta Australia Pty Ltd, Macquarie Park, NSW, Australia) at 0, 31.25, 62.5, 125, 187.5, 250, 500, 1000 g ha^{-1} for S and 0, 31.25, 62.5, 125, 187.5, 250, 500, 1000, 2000 g ha^{-1} for R, with or without the cytochrome P450 inhibitor piperonyl butoxide (PBO) at 2.1 kg ha^{-1} according to Preston *et al.*[22] The non-ionic surfactant BS1000 0.25% (*v/v*) was added to the herbicide treatment solution. PBO was applied 1 h prior to herbicide application. Herbicides were applied using a cabinet sprayer with a spray volume of 118 L ha^{-1} at a pressure of 200 kPa and a speed of 1 m s^{-1}. Plant mortality was determined 3 weeks after treatment. The median lethal dose (LD_{50}) (herbicide rate causing 50% plant mortality) values were estimated by non-linear regressing analysis using SigmaPlot® version 13.0 (Systat Software, Inc., San Jose, CA, USA). The data collected were fitted to the four-parameter logistic model:

$$y = C+(D - C)/[1 + (x/x_0)^b].$$

where C is the lower limit representing plant survival at infinitely large herbicide rates, D is the upper limit representing plant survival at low herbicide rates close to untreated controls, x_0 is the rate giving 50% plant response (LD_{50}) and b is the slope around x_0. Significant differences of estimated LD_{50} values and other data (i.e. metribuzin uptake and translocation and metabolism) between the R and S populations were determined by the *t*-test ($\alpha = 0.05$) using Prism® version 5.0 (GraphPad Software, Inc., San Diego, CA, USA). The R/S LD_{50} ratio was calculated to indicate the level of resistance.

2.3 Single-rate test for multiple herbicide resistance

Germinating seedlings of both R and S populations were transplanted into plastic trays (300 mm × 400 mm × 100 mm), with 50 seedlings per tray per treatment. The two- to three-leaf stage seedlings were treated with the following herbicides at highest Australian recommended field rates: diclofop-methyl (1500 g ha^{-1}, Diclofop-methyl 375 Herbicide, Imtrade Australia Pty Ltd, Kwinana, WA, Australia), clethodim (60 g ha^{-1}, Clethodim 240 Herbicide, Titan AG Pty Ltd, Newport, NSW, Australia), sulfometuron (37.5 g ha^{-1},750 WG Herbicide, Titan AG Pty Ltd, Belrose, NSW, Australia) and imazapic/imazapyr (36 g ha^{-1}, Onduty Herbicde, BASF Australia Ltd, Southbank, VIC, Australia). Plant mortality was recorded 21 days after treatment, and plants with active new growth were considered as survivors.

2.4 [^{14}C]-metribuzin uptake and translocation

For [^{14}C]-metribuzin (Institute of Isotopes Co. Ltd, Budapest, Hungary) uptake, translocation and metabolism studies, individual seedlings were transplanted into small plastic cups (60 mm × 60 mm) and kept in a growth chamber with 20 °C/15 °C day/night temperature, 12 h/12 h light/dark photoperiod and a photon flux density of 250 µmol quanta m^{-2} s^{-1}. When plants reached the two- to three-leaf stage, the first true leaf was marked and the metribuzin treatment solution ([^{14}C]-metribuzin mixed with metribuzin commercial formulation plus 0.25% (*v/v*) BS1000) was applied as a 1 µL droplet. Total concentration applied per plant was equal to 200 g metribuzin ha^{-1} with radioactivity of 0.93 kBq. Treated plants (seven from each R and S population) were harvested 24, 48 and 72 h after treatment. The treated leaf surface of each plant was rinsed in 20 mL of washing buffer containing 20% (*v/v*) methanol and 0.2% (*v/v*) Triton X-100 to remove unabsorbed [^{14}C]-metribuzin. Radioactivity in the leaf wash was quantified using a liquid scintillation counter (LSC) (Packard 1500, Tri-carb®; Perkin Elmer, Waltham, MA, USA). Roots of treated plants (seven) were rinsed in 50 mL washing buffer, and radioactivity in the root wash was quantified. Visualization and quantification of [^{14}C]-metribuzin uptake and translocation was performed according to Lu *et al.*[16]

2.5 [^{14}C]-metribuzin metabolism

The two-leaf stage R and S seedlings were treated with the treatment solution prepared as mentioned earlier, except that applied radioactivity per plant was 3.67 kBq to reduce the isotope dilution effect by plant tissue and maximize the ^{14}C signal-to-noise ratio for high-performance liquid chromatography (HPLC) analysis. The 1 µL droplet treatment solution was spread along the adaxial surface (close to the leaf base to facilitate translocation to the new growth) of the second fully expanded leaf of the plants. Treated plants were harvested 24, 48 and 72 h after treatment. The treated leaf of each plant was rinsed as described earlier. Plants were blotted dry, snap-frozen in liquid nitrogen, and stored at −80 °C until use. Five plants of each population were bulked as a replicate for each time point and three replicates per time point analyzed. Plant extraction and HPLC separation of metribuzin and its metabolites was performed using gradient reverse-phase HPLC equipped with a 600E dual-head pump with 717 plus autosampler (Waters, Milford, MA, USA) according to Lu *et al.*[16]

2.6 Sequencing of *psb*A, ACCase and ALS genes

Leaf tissue (about 100 mg) of individual survivors from the R population was used for DNA extraction. Bulked leaf material from S population without herbicide treatment was used as a control. The forward primer psbF3 (ATGACTGCAATTTTAGAGAGACGC) and reverse primer psbR1 (TAGAGGGAAGTTGTGAGCAT) were designed to amplify *Lolium rigidum* chloroplast *psbA* gene (980 bp) based on the *psbA* gene sequence of *Arabidopsis thaliana* (L.) Heynh. (GeneBank accession X79898.1). The ALS and ACCase gene were sequenced using published primers.[23–25]

3 RESULTS

3.1 Dose response to metribuzin and atrazine with and without the P450 inhibitor

Metribuzin at 150 g ha^{-1} controlled 65% of the S plants whereas it only controlled 10% of the R plants. Metribuzin at 300–600 g ha^{-1} was required to achieve control of the R population (Fig. 1). The LD_{50} values were 264 and 90 g ha^{-1} for R and S populations, respectively (Table 1), giving 2.9-fold resistance based on the R/S ratio of LD_{50}. Pre-treatment with the known cytochrome P450 inhibitor PBO affected dose response to metribuzin in R and S populations, reducing the LD_{50} value by 2- and 1.3-fold, respectively (Table 1). This indicates that the cytochrome P450 may be involved in metribuzin resistance in the R population.

In addition, atrazine at 250 g ha^{-1} achieved 85% control of the S plants whereas it only gave 30% control of the R plants (Fig. 2). The R population showed a higher LD_{50} to atrazine than the S (320 *versus* 114 g ha^{-1}) (Table 1), giving 2.8-fold resistance to atrazine. Pre-treatment with PBO affected dose response to atrazine in both R and S populations to a similar extent, reducing their LD_{50} values by about 1.5-fold (Table 1). This implies that the cytochrome P450 may not play a major role in atrazine resistance in this R population.

3.2 Resistance to ACCase- and ALS-inhibiting herbicides

Single-dose studies were conducted to screen for resistance to ACCase- and ALS-inhibiting herbicides in the R population. The S population was controlled by these herbicides whereas the R population showed resistance to ACCase-inhibiting herbicides diclofop-methyl and clethodim with 100% and 53% plant survival, respectively (Table 2). The R population also displayed resistance (> 80% survival) to ALS-inhibiting herbicides sulfometuron and imazamox/imazapyr mix (Table 2).

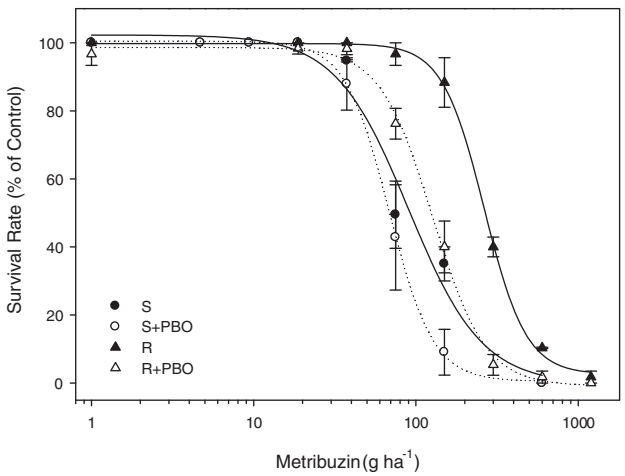

Figure 1 Dose response to metribuzin of the susceptible (S) and resistant (R) *Lolium rigidum* populations in the absence (filled symbols, solid lines) or presence (open symbols, dotted lines) of the cytochrome P450 inhibitor piperonyl butoxide (PBO).

3.3 Foliar uptake and translocation of [^{14}C]-metribuzin

Foliar uptake of [^{14}C]-metribuzin was similar in both R and S plants 48 and 72 h after treatment (Table 3), except for a small but significant difference 24 h after treatment. Similarly, translocation of metribuzin from treated leaf to untreated leaves, stem and roots was not significantly different between R and S plants (Table 3), as the majority of the radioactivity remained in the treated leaves, and only a small amount of [^{14}C] moved to other untreated parts of the plants. Phosphor imaging also revealed at all time points a very similar [^{14}C]-radioactivity distribution pattern between R and S plants (see Fig. 3 for representative images). Thus, it is evident that metribuzin resistance in this R population is not associated with differential metribuzin foliar uptake and translocation.

3.4 [^{14}C]-metribuzin metabolism

The conversion of [^{14}C]-metribuzin into polar metabolites in R and S plants was assessed 24, 48 and 72 h after treatment. Metribuzin and its metabolites were clearly resolved at approximate 35 min (peak 1), and between 10 and 25 min (peaks 2, 3 and unlabeled peaks), respectively, under our HPLC conditions (Fig. 4). A decrease in the metribuzin level over time was correlated with a concomitant increase in polar metabolites in both R and S plants (Table 4). However, the amount of radioactivity present as major metabolites of metribuzin was consistently higher (up to 5.3-fold) in R than in S plants at all three time points (Table 4). For example, 72 h after treatment, the percentage of parent metribuzin remained in the R was 20% lower than in S plants, corresponding to a 2.3-fold greater amount of major metribuzin metabolites (Fig. 4, peaks 2 and 3) in R than in S. These results demonstrate that R plants possess enhanced rates of metribuzin metabolism.

3.5 Sequencing of *psb*A, ALS and ACCase genes

The amplified 980 bp sequence encompasses the known PSII resistance-conferring mutation sites of the chloroplast *psb*A gene. Sequencing results of 16 individual R plants and the bulked leaf sample of five S plants revealed no nucleotide changes resulting in amino acid substitutions. Thus, the known mutations in the *psb*A gene conferring target site resistance to PSII-inhibiting herbicides were not present in the R population.

ALS gene sequencing of 22 individual R plants and the bulked leaf sample of five S plants revealed four previously known resistance mutations: Pro-197-Ser, Pro-197-Arg, Pro-197-Gln and Trp-574-Leu. Similarly, ACCase gene sequencing of 22 individual R plants and the bulked leaf sample of five S plants identified four previously documented resistance mutations: Ile-1781-Leu, Ile-2041-Asn, Ile-2041-Val and Asp-2078-Gly. At least two different mutant alleles were present in individuals of four clethodim-resistant plants.

4 DISCUSSION

PSII-inhibiting herbicides atrazine, simazine and metribuzin are widely used, but despite many years of use, resistance evolution to these herbicides in *Lolium rigidum* is still low.[26] In this current study, the R population was confirmed to have low level (about three-fold) resistance to metribuzin and atrazine, relative to the S population. Although the level of resistance measured under

Table 1 Parameter estimates of the non-linear regression analysis of the dose-response to metribuzin and atrazine for the susceptible (S) and resistant (R) *Lolium rigidum* populations in the absence or presence of the P450 inhibitor PBO (2100 g ha^{-1}) as a synergist

Treatment	Population	*D*	*C*	*b*	LD_{50} (g ha^{-1})	R^2
Metribuzin	S	102.3 (4.8)	−0.3 (1.0)	−1.9 (0.6)	90.4 (16.6)	0.99
	R	99.8 (0.9)	2.6 (1.6)	−3.4 (0.3)	264.0 (6.4)	0.99
Metribuzin +PBO	S	100.5 (0.5)	0.6 (0.8)	3.2 (0.1)	68.4 (0.89)	0.99
	R	98.6 (2.0)	−0.8 (2.3)	−2.7 (0.3)	126.6 (6.2)	0.99
Atrazine	S	100.8(6.5)	−3.4 (6.8)	−2.0 (0.5)	114.6(16.1)	0.99
	R	99.2 (0.7)	1.1 (1.0)	−4.0 (0.2)	319.5 (5.7)	0.99
Atrazine +PBO	S	97.1 (2.4)	1.4 (1.7)	−4.3 (0.6)	76.6 (3.2)	0.99
	R	98.8 (1.1)	0.3 (1.1)	−4.1 (0.3)	198.4(4.6)	0.99

Note: Standard errors are in parentheses.

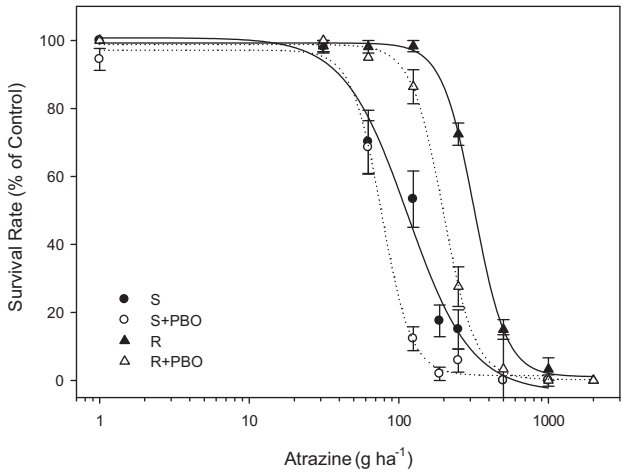

Figure 2 Dose response to atrazine of the susceptible (S) and resistant (R) *Lolium rigidum* populations in the absence (filled symbols, solid lines) or presence (open symbols, dotted lines) of the cytochrome P450 inhibitor piperonyl butoxide (PBO).

Table 3 Uptake and translocation of [^{14}C]-metribuzin applied to a single leaf of the susceptible (S) and resistant (R) *Lolium rigidum*

		Translocation (% absorbed)		
Population	Foliar uptake (% applied)	Treated leaf	Untreated leaf plus stem	Root
24 h				
S	66 (3.6)^a	90.2 (0.6)^a	4.3 (0.8)^a	5.5 (0.7)^a
R	54 (3.7)^b	88.9 (1.7)^a	3.0 (0.2)^a	8.1 (1.5)^a
48 h				
S	76 (4.2)^a	93.9 (1.0)^a	2.6 (0.3)^a	3.5 (0.7)^a
R	72 (3.5)^a	94.5 (0.9)^a	1.9 (0.2)^a	3.6 (0.8)^a
72 h				
S	78 (2.9)^a	96.5 (0.4)^a	1.6 (0.2)^a	1.9 (0.5)^a
R	75 (3.2)^a	96.8 (0.9)^a	1.1 (0.2)^a	2.1 (0.7)^a

Note: Standard errors are in parentheses. Means with the same letter in a column for each paired S and R populations at each time point are not significantly different ($\alpha = 0.05$) as determined by the *t*-test.

ideal glasshouse conditions is relatively low, it has significant consequences under field conditions where low herbicide rates are often encountered. High level resistance to PSII herbicides in weedy plant species is usually due to TSR mutations in the *psbA* gene (e.g. Ser-264-Gly).[27,28] Nevertheless, certain *psbA* mutations do confer low level resistance to PSII herbicides. For example, our recently reported *psbA* Phe-274-Val mutation in *R. raphanistrum* endows a low-level resistance to atrazine, metribuzin and diuron.[10] It is surprising that no resistance-endowing

Table 2 Percentage survival of the susceptible (S) and resistant (R) *Lolium rigidum* populations in response to herbicide of different modes-of-action

Herbicide	Rate (g ha^{-1})	Number of plants treated	Survival (%) S	Survival (%) R
Diclofop-methyl	1500	50	0	100
Clethodim	60	139	0	53
Sulfometuron	37.5	50	0	100
Imazamox+ imazapyr	24.75 + 11.25	139	0	80

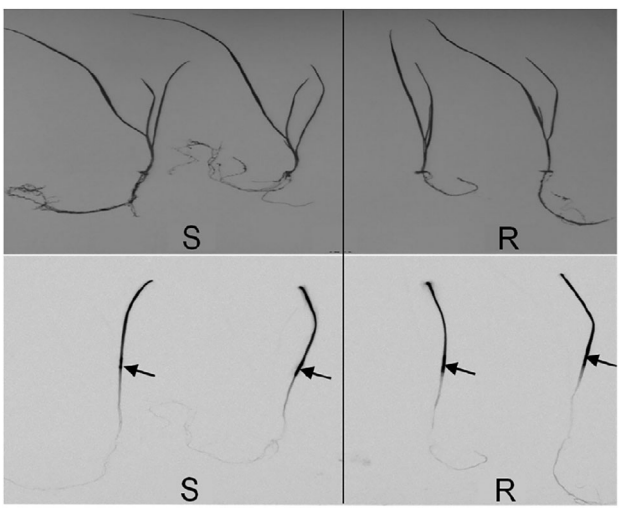

Figure 3 Representative camera (above) and phosphor (below) images showing [^{14}C]-metribuzin translocation in the susceptible (S) and resistant (R) *Lolium rigidum* plants 72 h after treatment. The arrow indicates herbicide application site.

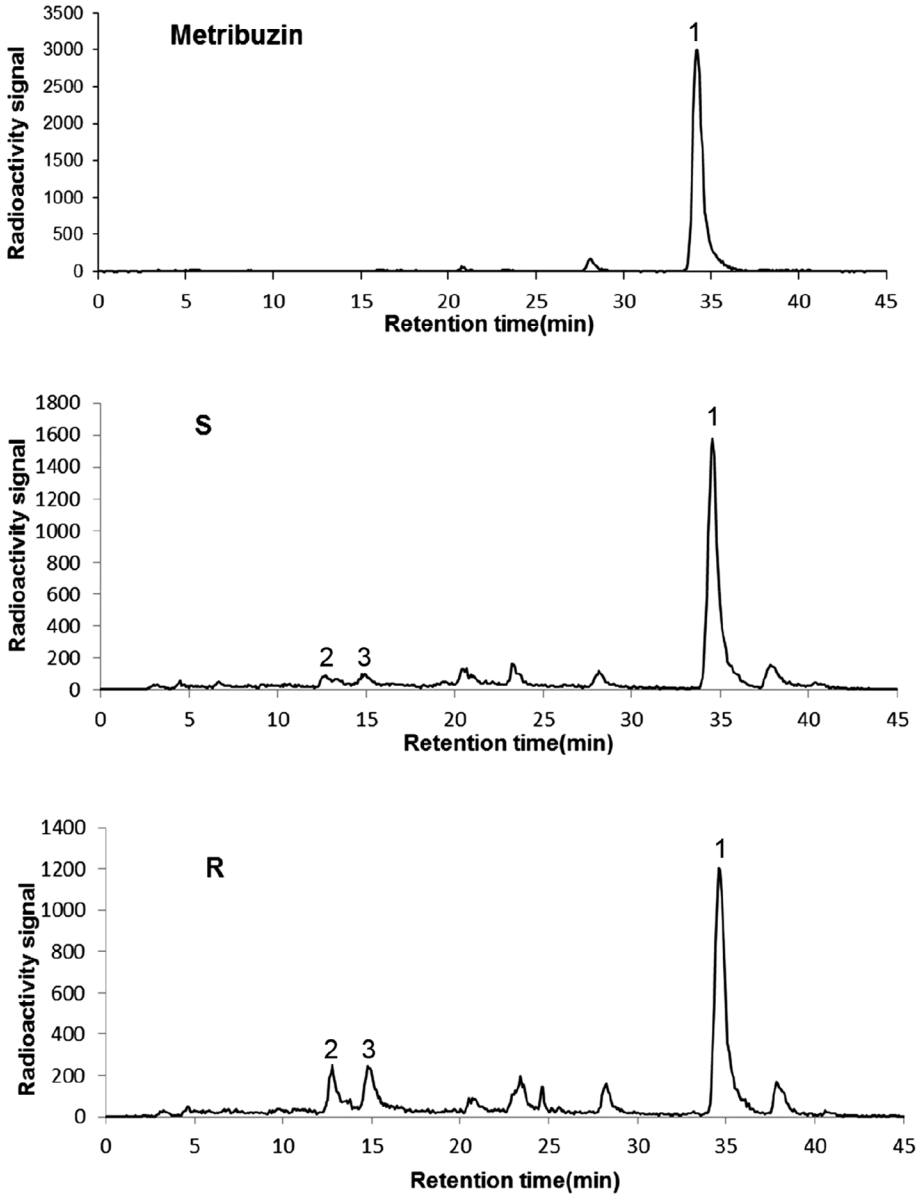

Figure 4 High-performance liquid chromatography (HPLC) chromatograms comparing the elution profiles of metribuzin (peak 1) and its metabolites (the major polar metabolite peaks 2 and 3 plus unlabeled peaks) in the susceptible (S) and resistant (R) *Lolium rigidum* plants, 72 h after treatment.

psbA gene mutations (either strong or weak) so far has been found in Australian *Lolium rigidum* given that all resistance mechanisms can be selected.

Herbicide metabolism is an effective NTSR mechanism. Metabolic herbicide resistance and cross-resistance are widespread in *Lolium rigidum*, *Alopecurus myosuroides* Huds., and increasingly prevalent in some other weed species.[11,13,29] In early studies by Preston and coworkers[21,22] enhanced metabolism capacity to PSII-inhibiting herbicides simazine and chlorotoluron in a *Lolium rigidum* population (VLR69) was demonstrated. Metabolic resistance to PSII-inhibiting herbicides was also reported in other weed species of *Abutilon theophrasti* Medic., *R. raphanistrum*, and *Amaranthus palmeri* S. Watson.[16,30,31] Here, we identify metabolic resistance to the PSII-inhibiting herbicide metribuzin in a *Lolium rigidum* population. Early studies reported in some plant species metribuzin can be metabolized to DK or DADK, or alternatively can be conjugated by *N*-glucoside followed by acylation to form a malonyl *N*-glucoside conjugate.[19,32,33] Diversity in metribuzin metabolite profile may be involved in differential crop susceptibility to metribuzin.[14–16] For example, the major metabolite of metribuzin was found to be DADK in susceptible soybean cultivars, whereas glucose conjugate was found in tolerant cultivars.[32] Due to a modest level of metribuzin metabolism in the R *Lolium rigidum* population the nature of metribuzin metabolites were not further identified in the current study. The ability of the P450 inhibitor PBO to reduce metribuzin (and to a less extent, atrazine) resistance in the R population (Table 1) suggests that metabolic resistance in *Lolium rigidum* likely involves cytochrome P450s.

In addition to resistance to PSII-inhibiting herbicides, the R population also showed multiple resistance to ACCase- and ALS-inhibitor herbicides due to target site mutations, this is expected because multiple resistance to ACCase and ALS inhibitor herbicides is very common in Australian *Lolium rigidum* populations,

Table 4 Metabolism of [^{14}C]-metribuzin by the resistant (R) and susceptible (S) *Lolium rigidum* plants 24, 48, and 72 h after treatment, as quantified by radioactive HPLC analysis

		Radiolabel (% of radioactivity recovered)		
Treatment duration	Population	Major metabolite (peak 2 in Fig.4)	Major metabolite (peak 3 in Fig.4)	Metribuzin
24 h	S	0.8(0.5)[a]	0.6(0.2)[a]	81(5.0)[a]
	R	2.8(0.4)[b]	3.2(0.7)[b]	82(1.6)[a]
48 h	S	4.4(0.6)[a]	2.9(0.9)[a]	76(0.4)[a]
	R	7.4(0.5)[b]	6.8(0.2)[b]	67(0.3)[b]
72 h	S	5.4(1.1)[a]	3.8(0.2)[a]	70(1.2)[a]
	R	9.7(1.0)[b]	11(0.5)[b]	58(1.3)[b]

Note: The proportion of the parent herbicide and metabolites was expressed as a percentage peak area of total radioactivity in the sample injection. Means with different letters in a column for each paired S and R populations at each time point are significantly different ($\alpha = 0.05$) as determined by the *t*-test.

and both TSR and NTSR to these herbicides are widely involved.[13,28,34] Although not examined in this study, metabolic resistance to ACCase- and/or ALS-inhibiting herbicides may also be involved in the R population studied, as was shown in other herbicide resistant *Lolium rigidum* populations in Australia.[11,13]

In conclusion, this is the first study that identifies metabolic resistance to the PSII-inhibiting herbicide metribuzin in a *Lolium rigidum* population. Together with our recent report on metabolic resistance to metribuzin in *R. raphanistrum*[16] we consider metabolic resistance to PSII-inhibiting herbicides is on the increase in Australian major weed species. More importantly, metabolic resistance selected by PSII and ALS inhibitors may potentially extend to herbicides of different modes of action [e.g. 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides], as demonstrated by Lu *et al*. in *R*. raphanistrum.[35] Hence, metabolic resistance needs to be managed effectively by diverse chemical and non-chemical methods such as herbicide mixture/rotation and harvest weed seed control, to preserve the valuable TT herbicide technology.

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