SONCHUS OLERACEUS RESISTANT TO THE ALS INHIBITING HERBICIDES

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Summary. A biotype of *Sonchus oleraceus* from Goondiwindi, Queensland, is the first dicot species in Australia resistant to ALS inhibiting herbicides. This biotype was selected for 8 years with chlorsulfuron and is resistant to chlorsulfuron and a wide range of other sulfonylurea and imidazolinone herbicides. The LD_{50} ratios for the resistant biotype are at least 60-fold greater for sulfonylureas and at least 4.5-fold greater for imidazolinones than for susceptible *S. oleraceus*. GR_{50} ratios are greater than 9-fold for sulfonylureas and 7.4-fold for imazapyr. The resistant biotype is not resistant to non-ALS inhibiting herbicides. This paper documents the dose response of the resistant *S. oleraceus* to a range of herbicides.

INTRODUCTION

Sonchus oleraceus is an annual weed present in all states of Australia and widespread in the temperate zones of both hemispheres (1). It can be a serious problem in the northern Australian cereal cropping zone.

Acetolactate synthase (ALS, also know as acetohydroxyacid synthase, or AHAS) catalyses the first reaction in the production of three branched chain amino acids, leucine, isoleucine and valine (2). This biosynthetic pathway is only found in microbes and higher plants. There are several classes of herbicides that inhibit ALS. Chlorsulfuron was the first ALS inhibitor herbicide in the sulfonylurea class, commercialised in 1982. Imidazolinone, triazolopyrimidine and pyrimidinyl thiobenzoates are three other herbicide classes that directly inhibit ALS activity (reviewed in 3).

In Australia, sulfonylureas are the most widely and frequently used herbicides throughout the cereal cropping zone, particularly chlorsulfuron and triasulfuron for control of *Lolium rigidum* (annual ryegrass) and many broadleaf weeds in cereals. To add to the selection pressure imposed by ALS inhibitors, the imidazolinone herbicide, imazethapyr, and two triazolopyrimidine herbicides, flumetsulam and metsulam are being marketed. These imidazolinone and triazolopyrimidine herbicides are recommended for weed control in some dicot crops. Until recently these crops were an important break from sulfonylureas, but now there will be an ALS-inhibitor herbicide that could be used in each part of the cereal-legume rotation. This can only lead to a higher selection pressure imposed on all susceptible weed species.

In North America, eight dicot and two grass weeds have developed resistance to ALS inhibitors (reviewed in 3). In contrast, until now annual ryegrass has been the only weed to develop resistance to the ALS inhibitors in Australia (4, 5). However, our current work reveals that biotypes of three dicot weeds, *S. oleraceus*, *Sisymbrium orientale* and *Brassica tournefortii*, have developed resistance to the ALS inhibitors following selection with sulfonylurea herbicides. This study reports the first broadleaf weed in Australia, *S. oleraceus*, to become resistant to ALS inhibitors.

METHODS

Plant material. Seed of *S. oleraceus* was collected in the summer of 1991 from a wheat field near Goondiwindi in which chlorsulfuron had failed to control this species. In this field chlorsulfuron was applied between 10.5 and 12.75 g a.i./ha, either pre- or post-emergent consecutively for 8 years. Hereinafter this population is referred to as the resistant biotype. Susceptible seed (hereinafter referred to as the susceptible biotype) was collected from the Waite Institute from a paddock which had never been treated with an ALS inhibitor.

Pot experiments. Seeds were germinated on 0.6% agar in a seed incubator. After five days, seedlings were transplanted into 17 cm pots outside. When at the 3-4 leaf stage, the plants were sprayed in a precision herbicide spray cabinet. Both biotypes were tested post-emergent with six ALS inhibitor herbicides and five herbicides with different modes of action. There were five replicates (pots) per rate and seven rates per herbicide. The herbicides tested were chlorsulfuron, sulfometuron, metsulfuron, imazapyr, flumetsulam, bromoxynil, diuron, metribuzin, MCPA and diflufenican. Surfactant (0.2% (v/v) Agral 600®) was applied with each herbicide.

After herbicide application, the plants were returned to the field and organised in a completely randomised design. Thirty days after spraying, survival was recorded and dry weight 48 h later. The experiment was repeated and the data pooled for analysis.

RESULTS AND DISCUSSION

Resistance to ALS inhibitors. The pot studies confirmed the field failure of chlorsulfuron in that the *S. oleraceus* biotype is highly resistant to this herbicide. Susceptible plants were killed with 2.5 g a.i./ha whereas doses above 67.5 g a.i./ha chlorsulfuron were required to kill resistant plants (Fig. 1a). Although the difference in dry weight response between the resistant and susceptible biotypes was not as large as the difference in plant survival to chlorsulfuron, the difference was still significant (Fig. 1a and 1b). The large difference in response to chlorsulfuron between both biotypes is further exhibited by the R/S *LD_{50} ratio, being greater than 86 (Table 1) and the R/S #GR_{50} greater than 28 (Table 1). This *S. oleraceus* biotype was also tested with two other sulfonylureas and found to be highly resistant (Table 1). Very similar GR_{50} ratios have been recorded for *Kochia scoparia* in North America (6). However, for resistant biotypes of *Stellaria media*, *Lolium perenne* and *Salsola iberica* higher GR_{50} ratios were recorded suggesting that plant growth reduction was not as sensitive to these sulfonylurea herbicides as for this *S. oleraceus* biotype (7).

The finding that the *S. oleraceus* biotype is highly resistant to sulfometuron-methyl (Table 1) indicates that the mechanism of resistance to the ALS inhibitors may be a mutant form of the ALS enzyme, as has been documented for all dicot weed species that have developed resistance to the ALS inhibitors in North America (3). This indication is based on work that has shown that sulfometuron-methyl metabolism is very slow, even in species that rapidly metabolise other sulfonylureas such as wheat (8, 9). In light of such findings, because the *S. oleraceus* biotype is highly resistant to sulfometuron, then resistance is unlikely metabolism based, but most likely due to a mutant ALS enzyme. Enzyme assays have not yet been performed.

The *S. oleraceus* biotype is less resistant to imidazolinone herbicides. This is illustrated by the LD_{50} ratios which are at least 10-fold greater for the sulfonylurea herbicides (Table 1). The GR_{50} ratio of the resistant *S. oleraceus* falls within the published results of the GR_{50} ratios of three ALS target-site mutant resistant weed species; the GR_{50} ratio of *S. media* was 8-fold greater, *L. perenne* 600-fold greater and *S. iberica* 2.5-fold less (7).

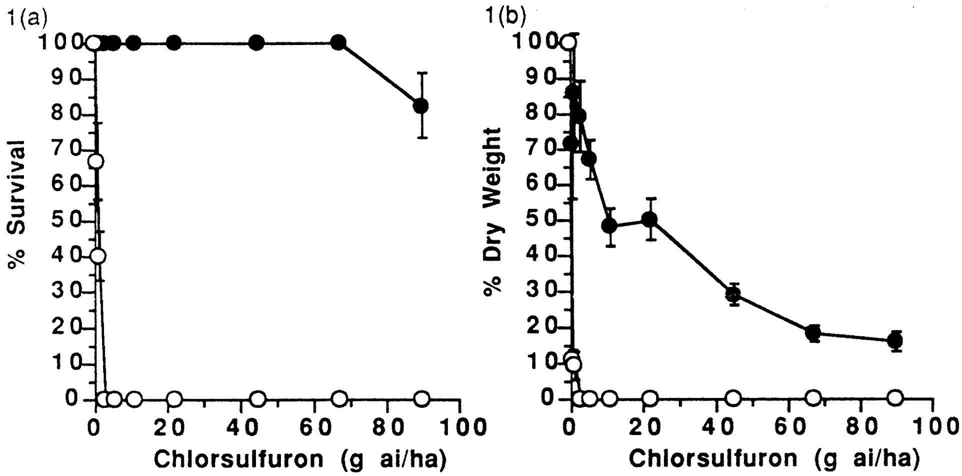

Figure 1. (a) Percent survival and (b) percent dry weight of susceptible (O) and resistant (●) *S. oleraceus* to chlorsulfuron 30 days after treatment. Each point is the mean of 5 replicates repeated twice. Error bars show the standard error of the mean for that rate.

Table 1. LD_{50} and GR_{50} measurements for resistant and susceptible *S. oleraceus* treated with 11 herbicides

Herbicide	LD_{50} (g a.i./ha)			GR_{50} (g a.i./ha)		
	R	S	R/S ratio	R	S	**R/S ratio**
Sulfometuron	>120	<0.94	>128	26.3	<0.94	**>28**
Chlorsulfuron	>90	1.1	>82	16.6	<0.71	**>23**
Metsulfuron	>24	<0.38	>63	3.3	<0.38	**>9**
Imazapyr	142	17	8.4	32.7	4.4	**7.4**
Imazethapyr	467	105	4.5	-	-	-
Flumetsulam	>160	>160		>160	>160	
Metribuzin	31.6	29.7	1.1	25.6	33.9	**0.8**
Diuron	24.8	35.7	0.7	15	29.1	**0.5**
Bromoxynil	8.9	8.1	1.1	-	-	-
Diflufenican	52.1	46	1.1	13	17.5	**0.7**
MCPA	275	256	1.1	77	87	**0.9**

* LD_{50} is defined as the rate of herbicide application in grams active ingredient/hectare (g a.i./ha) required to kill 50% of the population.

' GR_{50} is defined as the rate of herbicide application in g a.i./ha required to inhibit plant growth by 50% relative to untreated controls.

Flumetsulam had no activity on either *S. oleraceus* biotype. This finding is consistent with technical information supplied by Dow Elanco. In contrast, susceptible *S. media* was sensitive to DE-489 another triazolopyrimidine, whereas the chlorsulfuron resistant *S. media* biotype was cross-resistant to DE-489 (10). It will be interesting to test if ALS from *S. oleraceus* is inhibited by flumetsulam.

Both *S. oleraceus* biotypes were equally sensitive to herbicides with modes of action different from ALS inhibitors (Table 1). This response was also documented for resistant and susceptible *K. scoparia*, tested with bromoxynil, MCPA and diuron (6). Therefore, as for the North American ALS inhibitor resistant dicot weed species the resistant *S. oleraceus* does not show any cross resistance to non-ALS inhibitor herbicides.

CONCLUSION

This study documents the first dicot weed species, *S. oleraceus* resistant to the ALS inhibiting herbicides in Australia. This biotype is highly resistant to the sulfonylurea herbicides, similar to the ALS inhibitor resistant dicot weeds of North America and is resistant, but less so, to the imidazolinone herbicides. Early indications suggest that resistance is probably due to a mutated ALS enzyme, although this has not yet been confirmed. Current studies have recently confirmed two other dicot species as highly resistant to sulfonylureas. There seems little doubt that other weed species will develop resistance to the ALS inhibitor herbicides. Given the widespread usage of sulfonylureas and the introduction of new ALS inhibitors it is inevitable that further species will develop resistance. All sectors of the industry should address this looming problem.

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