Extent of herbicide resistant common sowthistle (*Sonchus oleraceus*) in southern Australia

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Summary Herbicide resistant weeds are a major problem in crop production. Random weed resistance surveys are a tool to determine the extent of this problem. In Australia, common sowthistle (*Sonchus oleraceus* L.) has been identified as resistant to ALS inhibiting herbicides, synthetic auxin and glyphosate. Resistance surveys in the southern region show that resistance to ALS inhibitors is widespread. In the Midnorth, Mallee and Yorke Peninsula of South Australia and the Northeast, Wimmera and Mallee regions of Victoria, over 80% of randomly collected samples have exhibited resistance to sulfonylurea herbicide chlorsulfuron. Common sowthistle has also been screened with the synthetic auxin herbicide 2,4-D with a low frequency of resistance detected. No resistance to EPSPS inhibitor glyphosate or the PSII inhibitor atrazine has been detected in the southern region.

Of the samples screened to both sulfonylurea and imidazolinone ALS inhibitors, 57% were resistant to both chemical families. These results indicate a high incidence of resistance to both sulfonylureas and imidazolinones in common sowthistle. A significant proportion of lentils grown in the southern region are ALS inhibitor tolerant varieties and growers rely heavily on these herbicides to control broadleaf weeds in this crop. Losing control of common sowthistle using this chemical group will be a major weed management challenge.

Keywords ALS inhibitor resistance, cross-resistance, herbicide resistance monitoring surveys.

INTRODUCTION

Weeds negatively impact global crop production by reducing yields, affecting grain quality or serving as alternate hosts to insect pests or disease vectors. Many weed species have evolved resistance to herbicides, making them an even bigger threat to sustainable crop production (Powles and Yu 2010). In southern Australia, common sowthistle (*Sonchus oleraceus* L.) control is an emerging issue, particularly in weakly competitive broadleaf crops such as lentils. The shift to reduced tillage practice in Australia has accelerated resistance to several herbicide modes of action commonly used to control it. ALS inhibitor resistance in common sowthistle was first discovered in 1991 in a population from Goondiwindi in Queensland (Boutsalis and Powles 1995) but has since been discovered in South Australia (SA) and Victoria. More recently, glyphosate and synthetic auxin resistance have been detected in New South Wales and southern Australia, respectively (Heap 2018).

Random weed resistance surveys are an important tool for monitoring the distribution of herbicide resistant weeds over time. In southern Australia, resistance surveys have been conducted regularly since 2005, covering each of the major cropping regions in South Australia and Victoria on a five year rotation. Common sowthistle samples have been collected and screened through this program since 2010, and the results have indicated widespread ALS inhibitor resistance and emerging, low-frequency synthetic auxin resistance.

MATERIALS AND METHODS

Field surveys This study is based on common sowthistle data from monitoring surveys conducted from 2010–2016. Surveys were timed to coincide with crop and weed maturity (but before harvest) for the given region that year. Cropping paddocks were chosen at random and surveyed as described in Boutsalis *et al.* (2012). Mature common sowthistle seed was collected and pooled from plants within a paddock to form a representative sample of the population. Seed was collected in labelled paper bags, air dried, and stored at ambient temperature until screening.

Herbicide screening Weed samples were grown and screened at the University of Adelaide Waite Campus in Urrbrae, SA the autumn/winter following their collection (approximately six months after maturity). A small number of seeds of each sample were sown in pots on the surface of a coco-peat potting mix (Boutsalis *et al.* 2012) for germination and then transplanted at the first-leaf stage. In earlier surveys (2010–2013), as many seedlings as were available for each sample were screened (average = 15 ± 11 plants). In subsequent years the protocols were standardised to 10 plants per sample in 0.9 L pots at a density of 5 plants per pot (435 plants m^{-2}). After transplanting,

plants were left outdoors and watered as needed. Herbicide application was conducted at approximately the 4–5 leaf stage using a laboratory moving boom cabinet sprayer equipped with TeeJet flat fan (015-110) nozzles and an output of 118 L ha^{-1}. Herbicide screening rates (Table 1) are based on previously established discriminating doses between susceptible (S) and resistant (R) plants and, in most cases, are close to the field rate of the herbicide.

Data analysis A sample was considered to be from a resistant population if at least 20% of plants survived the screening rate. Resistance levels in different areas of southern Australia were compared by grouping data points into nine regions and comparing the proportion of resistant samples from each region. Confidence intervals were calculated using a one-proportion z-test. For regions with n<30, an exact binomial test was used. Proportions of samples for which confidence intervals did not overlap were considered to be significantly different. Temporal comparisons between cropping regions were done by two-proportion z-test. A Chi-squared test was used to test for independence of sulfonylurea and imidazolinone resistance. All statistical analyses were performed using R statistics package (R Core Team 2018). Survey data were mapped and analysed for spatial autocorrelation (Moran's I) using ArcMap 10.5.1.

RESULTS

None of the samples tested were resistant to glyphosate or atrazine (Table 1). In 2014, screening with 2,4-D identified four resistant samples (2%). One of these was from the Eyre Peninsula (34.3680, 135.3638), while the other three were from southwest Victoria near Shelford (8.0153, 143.9238), Foxhow (38.0196, 143.4063), and Nerrin Nerrin (37.7787, 142.9747). Most of these exhibited low-level resistance (20% survival), except the sample from Nerrin Nerrin (50% survival). The Eyre Peninsula and the Nerrin Nerrin sample both had high levels (>90% survival) of resistance to sulfonylureas.

Analysis of the survey results showed that ALS inhibitor resistance to both the sulfonylureas (SU) and the imidazolinones (IMI) is common in southern Australia (Table 1). Available SU resistance data for most major cropping regions shows that resistance is very common (>65% of samples) in all regions (Figure 1A). In most cases the proportions from different regions were not significantly different from each other. Available IMI resistance data from 2015 and 2016 for surveys done in Victoria (Figure 1B) suggests that IMI resistance in Victoria could be just as common. Among samples screened with an SU and an IMI, more than half (57%) were resistant to both chemical families and few were susceptible to both (13%) (Figure 2). A Chi-square test of independence between SU resistance and IMI resistance indicated that resistance to the two chemical families was not independent (P = 0.02553).

The major cropping regions of Victoria have now been surveyed twice at 4–5 year intervals, allowing the analysis of resistance shifts over time (Table 2). Most areas returned a higher percentage of resistant samples in the more recent survey compared to the earlier ones; however, none of these differences were statistically significant. Interestingly, the only statistically significant difference was a decrease in the proportion of resistant samples from 2011–2016 in Northeast Victoria.

The spatial relationships between the data were examined by testing for spatial autocorrelation (Moran's I) of SU resistance. The percentage of plants surviving chlorsulfuron application in each sample was used as the input and returned a Moran's Index of 2.029035 and a P-value of 0.001161, indicating that clustering had not occurred due to random chance.

Table 1. History of herbicide screening, rate, sample size and total percentage of resistant samples identified from all years of screening for each herbicide.

Herbicide	Survey years	Rate (g ai ha^{-1})	n	Resistance (% samples)
Chlorsulfuron	2010–2016	15	355	78
2,4-D	2013–2016	455	169	2
Glyphosate	2013–2016	300	169	–
Atrazine	2014, 2015	900	53	–
Imazamox + imazapyr	2015, 2016	24.75 + 11.25	84	68

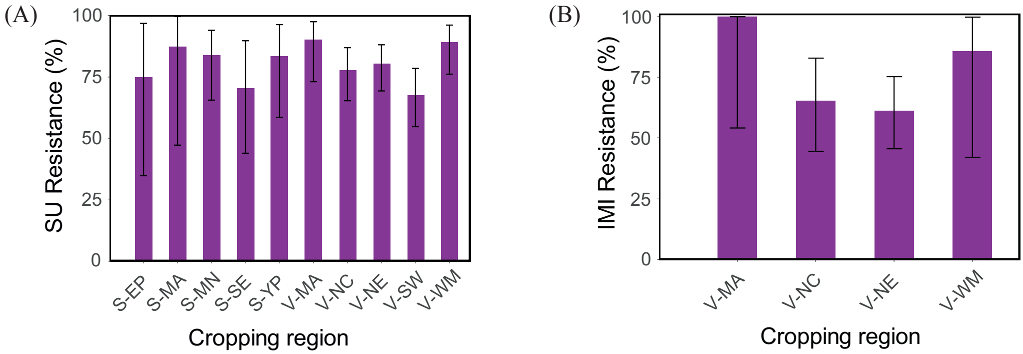

Figure 1. Percentage of populations resistant to (A) sulfonylurea (SU) and (B) imidazolinone (IMI) herbicides are shown by cropping region in South Australia (S–) and Victoria (V–). Cropping regions are the Eyre Peninsula (S-EP, SU n = 8), the SA Mallee (S-MA, SU n = 8), the Mid-north (S-MN, SU n = 31), the Southeast (S-SE, SU n = 17), the Yorke Peninsula (S-YP, SU n = 18), the Victorian Mallee (V-MA, SU n = 31, IMI n = 6), North-central (V-NC, SU n = 63, IMI n = 26), Northeast (V-NE, SU n = 76, IMI n = 44), and Southwest (V-SW, SU n = 65) Victoria, and the Wimmera (V-WM, SU n = 47, IMI n = 7). Errors bars represent 95% confidence intervals.

Table 2. Comparison between Victorian cropping regions with repeat survey data. P-values are the result of two-proportion z-tests.

Survey	n	Resistance (% samples)	P
Southwest			
2010	47	66	0.8517
2014	18	72	
North-central			
2010 + 2011	33	79	1
2015 + 2016	30	77	
Mallee			
2010	19	84	0.4095
2015	12	100	
Wimmera			
2010	34	85	0.3504
2015	13	100	
Northeast			
2011	30	97	0.009141
2016	46	70	

DISCUSSION

Herbicide resistance monitoring surveys are a valuable monitoring tool for growers, advisors and researchers. The resistance surveys in southern Australia have shown that common sowthistle has widespread resistance to ALS inhibitor herbicides. It is also clear that cross-resistance between the sulfonylurea and imidazolinone herbicides occurs widely. If current weed management practice does not effectively adapt to this fact, there will be serious consequences for the sustainability of lentil production in Australia.

When analysed by cropping region, the current dataset results in relatively low sample sizes for some areas which make it difficult to identify significant differences between regions. As more data is added (from the SA Mallee and Southeast survey in late 2017, and the Mid north and Yorke Peninsula in late 2018), analytical power of this dataset will continue to increase. However, the magnitude of the problem in general is still apparent, especially for the sulfonylureas. In the same program of surveys in southern Australia, sulfonylurea herbicides were also the most frequent case of resistance for rigid ryegrass (*Lolium rigidum* Gaudin) (Boutsalis *et al.* 2012). The frequency of resistance in this dataset was relatively high when compared to surveys of other weeds from other cropping areas around the world (Beckie *et al.* 2008).

The fact that SU and IMI resistance failed a test of independence is an indicator of cross-resistance between these two chemical families. It is well known that ALS inhibitor resistance can be conferred by a target-site mutation in the ALS gene, and at least five such mutations have been established in resistant weeds (Tranel and Wright 2002). Different mutations may confer a different profile of resistance across chemical families of ALS inhibiting herbicides and the pattern of resistance seen in the survey (Figure 2) could be the result of these differing mutations.

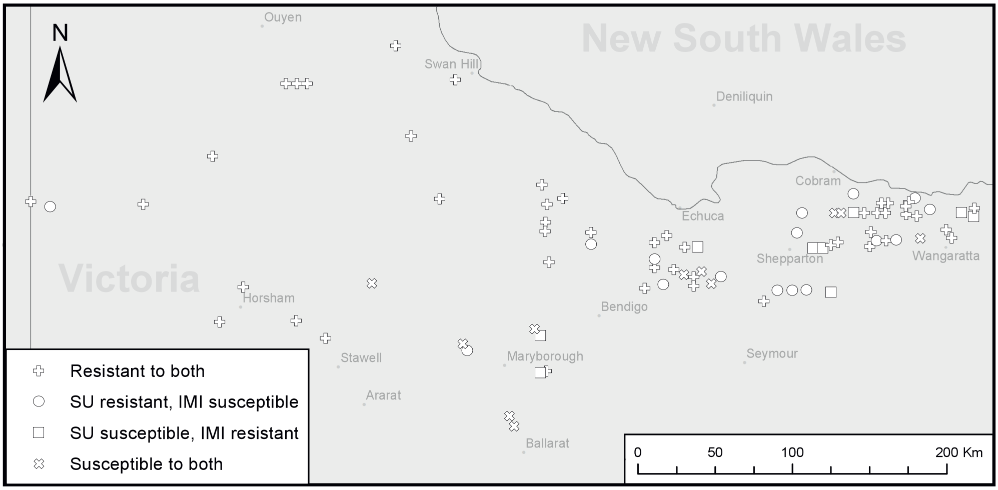

Figure 2. Map showing the distribution of ALS inhibitor resistance in regions of Victoria surveyed in 2015 and 2016. Resistance to both the sulfonylurea (SU) and imidazolinone (IMI) chemical families was present in 48 samples (✙), 16 samples were SU resistant and IMI susceptible (○), 9 samples were SU susceptible and IMI resistant (□), and 11 samples were susceptible to both chemical families (⛝).

While the frequency of resistance did increase from the 2010–2011 round of surveys to the 2015–2016 round, most of these differences were not significant (Table 2). Again, larger sample sizes as surveys continue will help to elucidate these differences. During the later (2015–2016) surveys, there was increased interest in screening roadside populations of common sowthistle and this could have influenced the proportion of resistant samples.

The presence of spatial correlation in SU resistance is an indication of gene flow. Common sowthistle is a wind-dispersed weed capable of travelling long distances (Hutchinson *et al.* 1984), but the extent to which this phenomenon contributes to resistance gene flow is not well understood. Data from this survey indicate that it could be a relevant factor and present the possibility of using ALS mutations as a marker to study gene flow. The results of the survey indicate that further investigations into the biology and ecology of common sowthistle will be crucial to the development of effective management strategies that will help to maintain the sustainability of lentil production systems.

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