

## Genetic diversity among ALS-inhibiting herbicide resistant and susceptible populations of *Sonchus oleraceus* L. (sowthistle) in Australia

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**Summary** *Sonchus oleraceus* L. (sowthistle) is a widespread weed in Australia. It is a weed of bare ground and to a lesser extent grain cropping. This means it is a problem in fallows in Queensland and northern New South Wales cropping systems. In 1991 the first *S. oleraceus* resistant to acetolactate synthase (ALS) inhibiting herbicides was found at Goondiwindi in Queensland and since then numerous resistant *S. oleraceus* have been identified in Queensland and northern New South Wales. Resistance may have evolved or been transported to other locations in Australia. *S. oleraceus* is self-pollinated and produces wind-dispersed seeds suggesting resistant *S. oleraceus* could easily spread. To elucidate patterns of resistance in Australia, seed from individual plant accessions was collected across many sites from all states in Australia. Seedlings were treated with an ALS-inhibiting herbicide to determine whether resistance was present. For accessions collected in New South Wales, Queensland, South Australia, Western Australia, Victoria and Tasmania, resistance frequencies were 78%, 59%, 29%, 17%, 8% and 0%, respectively. Resistance is widespread in Australia. AFLP analysis was used to investigate the genetic diversity of genotypes. A dendrogram produced from 493 scored loci across populations displayed large genetic diversity across Australia. Genetic diversity was apparent within states and even individual fields. DNA samples from a single field were used to deduce whether localised resistance was the result of a single mutation. This research demonstrates *S. oleraceus* has evolved resistance to ALS-inhibiting herbicides on multiple occasions in Australia.

**Keywords** Sowthistle, *Sonchus oleraceus*, genetic diversity, AFLP analysis, ALS-inhibiting herbicide resistance.

### INTRODUCTION

Agricultural weeds evolve in response to crop cultivation (Neve *et al.* 2009). Herbicide resistance threatens the efficiency and profitability of agricultural enterprises worldwide. Herbicide resistance evolves as a result of selection for individuals within weed species that can survive the normal rate of herbicide

application. Resistant alleles occur at low frequencies within natural populations (Preston and Powles 2002), but frequent use of herbicides with the same mode of action selects for these resistant alleles. Ashigh and Tardif (2007) report that the target site for resistance to ALS-inhibitors in weed species is caused by substitution at one of six conserved locations: Ala<sub>122</sub>, Pro<sub>197</sub>, Ala<sub>205</sub>, Asp<sub>376</sub>, Trp<sub>574</sub> and Ser<sub>653</sub>.

*Sonchus oleraceus* is self-pollinated and seed movement is the only avenue for resistance alleles to enter a population or colonise new areas. Seed dispersal also influences how rapidly resistance spreads. For example, wind blown *Lactuca serriola* L. seed has been associated with its rapid dispersal over large distances (Lu 2005).

*Sonchus oleraceus* is a wind-dispersed weed having achenes with a pappus enhancing dispersal so may also be readily dispersed across large areas by wind movement. Seeds have also been reported to have been dispersed by water and birds (Holm *et al.* 1977, Hutchinson *et al.* 1984, Andersson 1991). *S. oleraceus* seed has little dormancy and readily germinates (Widderick 2002), so the gene pool changes rapidly enhancing the development of resistant populations when selection pressure is present.

Herbicides are effective in controlling this weed, but in 1991 the first *S. oleraceus* resistant to ALS-inhibiting herbicides was recorded in Australia (Boutalis and Powles 1995). Little is known about wind dispersal of the ALS-inhibiting resistance gene through seed movement. An understanding of the evolution and dispersal of this resistance gene is likely to be helpful in not only managing resistant *S. oleraceus*, but also in understanding resistance to other herbicides in self-pollinated and wind-dispersed weeds.

Through knowledge of the major mechanisms of gene flow from plants resistant to ALS-inhibiting herbicides, management options can be manipulated to reduce the rate of resistance increase. For example, Walker *et al.* (2005) advocate spraying small seedlings and controlling late flushes of *S. oleraceus* in winter crops with selective herbicides instead of waiting for the first fallow spray after harvest. These strategies will aid in reducing plant numbers prior to flowering,

reducing the possibility of gene flow through seed set. This may not be the only method of controlling gene flow and understanding the mechanisms of gene flow can assist in selecting the most effective weed control management methods.

*Sonchus oleraceus* is a weed of major importance in the northern Australian cropping regions and the evolution of ALS-inhibiting herbicide resistance complicates management of this weed. This work researched the evolutionary biology and gene flow of *S. oleraceus* with a view to developing improved management strategies to minimise adaptation to herbicides and subsequent spread of resistant genotypes. The aim of this work was to determine how widespread resistance was in Australia and to use AFLPs to elucidate the relationship of resistant genotypes in Australia and provide an opportunity for improving current management practices.

#### MATERIALS AND METHODS

Seeds from individual plant accessions were collected throughout Australia between 1997 and 2008. One to five capitulum seed heads were collected from each plant and the location recorded using a global positioning system (GPS).

Seedlings were germinated from the collected seed. Leaf material was collected from one seedling from each of the seed samples and used for DNA extraction. Seedlings were then treated with 15 g a.i. ha<sup>-1</sup> chlorsulfuron. The herbicide was applied in a custom-built spray cabinet through two flat-fan nozzles on a moving boom 40 cm above the plants. The nozzle output was 103 L ha<sup>-1</sup> at a pressure of 240 kPa with a boom speed of 1 m s<sup>-1</sup>. Thirty days after treatment plants were scored as alive (resistant) or dead (susceptible).

DNA was extracted using the DNeasy® Plant Mini Kit (QIAGEN, cat#69106) and used for AFLP analysis. The AFLP technique described by Vos *et al.* (1995) was modified for use with fluorescent detection. The DNA was cut using *Mse*I and *Pst*I restriction enzymes, consequently *Mse*I and *Pst*I adapters were used in the ligation step. Pre-amplification PCR used *Pst*I+A and *Mse*I+C with the selective PCR amplification using dimers of *Pst*I+A and *Mse*I+C sequences (*Mse*I+CC and *Pst*I+AC; *Mse*I+CT and *Pst*I+AG). *Pst*I+AC and *Pst*I+AG dimer primers were fluorescently labelled. PCR products were run on an Applied Biosystems 3730, fluorescence-based DNA analyser at the Australian Genome Research Facility (AGRF) in Adelaide. AFLPs are used in population genetics for genetic variation analysis as they are rapid to develop, produce individual fingerprints and use low amounts of DNA for analysis (Vos *et al.* 1995).

Genomic data were viewed with GeneMapper® software for the presence or absence of peaks which were then analysed using PopGene® software to determine genetic relationships.

Two polymerase chain reaction (PCR) forward (TCCTCGTCTGAAGCCCTCGAGC) and reverse (CAAGCTGCTGCTGAATATC) primers were used to amplify a section of the ALS gene known to contain the region where mutations conferring chlorsulfuron resistance occur from each of the 22 *S. oleraceus* biotypes.

#### RESULTS AND DISCUSSION

Of the 274 *S. oleraceus* individuals tested, 121 were resistant to chlorsulfuron and 153 were susceptible giving a total of 44% resistance to the ALS-inhibiting herbicide, chlorsulfuron (Table 1).

Table 1 shows that resistance is widespread in Australia. Higher levels of resistance are noted in the northern cropping areas of Australia in New South Wales (NSW) and Queensland. This suggests that the high intensity cropping practices in these areas have produced higher levels of resistance. It should be noted that one accession from Tasmania is not sufficient to provide meaningful data concerning the resistance level in Tasmania, but did provide DNA for genetic comparison purposes.

A dendrogram was determined using 493 scored loci (data not shown). Despite some evidence of geographic clustering, genetic diversity was apparent within states and even individual fields. Many different resistant genotypes were identified, although similar resistant genotypes were detected in Victoria and Queensland.

The sequencing data for a single field showed that two individuals had the same mutation and were closely located in the field. A further two individuals were resistant but mutations were not identified in the part of the gene sequenced with the primers used, suggesting a mutation at an unknown location.

**Table 1.** ALS-inhibiting herbicides susceptibility (Sus) and resistance (Res) of 274 *S. oleraceus* plants collected in Australia.

Australian states	Total	Sus	Res	% Res
NSW	32	7	25	78
Queensland	98	40	58	59
South Australia	119	84	35	29
Western Australia	12	10	2	17
Victoria	12	11	1	8
Tasmania	1	1	0	0
Grand total	274	153	121	44

*Lactuca serriola* has seed architecture similar to *S. oleraceus* and is also self-pollinated. Lu *et al.* (2007) investigated genetic relationships over large areas for *L. serriola*. This study reports seed movement over distances of up to 43 km. Thus, dispersal patterns from *S. oleraceus* seed could be expected to be similar to that of *L. serriola*.

The herbicide testing indicates widespread resistance to chlorsulfuron in *S. oleraceus* in Australia and the AFLP and sequencing results reported here indicate a high level of genetic diversity in this species across Australia as well as a high number of different resistant genotypes. Similar genotypes were observed in both Queensland and Victoria and although seed can move great distances via wind-dispersal, it is unlikely that resistant genotypes would be moved that far over a relatively short time period. Therefore, it is most likely that resistance has evolved, or been selected for, in several different genotypes across the continent at different times through the persistent use of ALS-inhibiting herbicides. Additional sequencing work, not reported here, looked at whether resistant populations all had the same mutations in the ALS gene and whether there was a geographical correlation in identified mutations in Australian plants. However, different mutations, some occurring in the same geographical location were identified in the resistant plants. This supports the supposition that *S. oleraceus* has evolved resistance to ALS-inhibiting herbicides as a result of independent mutation events on multiple occasions in Australia (R.S. St John-Sweeting unpublished data).

It is possible, particularly for a weed with wind blown seeds, for resistance to spread across a landscape. The research here demonstrates that while seed movement can spread resistance, independent evolution of resistance is as important or more important than spread at the landscape level. A similar finding has also been reported for *L. serriola*, another species with wind blown seed (Lu *et al.* 2007). Spread of resistance by seeds may be more important at a farm level and producers may need to introduce management strategies to manage this problem.

ALS-herbicide resistance in *S. oleraceus* is now present in a number of states in Australia with resistance evolving from a number of independent mutation events. This information coupled with knowledge of weed biology and ecology further supports the need for integrated weed management strategies to manage this species. For example *S. oleraceus* is a self-pollinated species and this study emphasises the need for farmers to have a zero tolerance approach to localised herbicide resistant infestations and use alternate control methods if a resistant population is detected or suspected.

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