

Gene movement in herbicide resistant sowthistle (*Sonchus oleraceus* L.)

Robin S. St. John-Sweeting^{1,4}, Christopher Preston^{1,4}, Jeanine Baker^{2,4}, Steve Walker^{3,4} and Michael Widderick^{3,4}

¹ School of Agriculture, Food and Wine, University of Adelaide, PMB1, Glen Osmond, South Australia 5064, Australia

² Bureau of Rural Sciences, Invasive Species Section, GPO Box 858, Canberra, ACT 2601, Australia

³ Department of Primary Industries and Fisheries, PO Box 2282, Toowoomba Queensland 4350, Australia

⁴ CRC for Australian Weed Management

Email: robin.stjohn-sweeting@adelaide.edu.au

Summary Sowthistle (*Sonchus oleraceus* L.) is a weed of both bare ground and grain cropping and is widespread worldwide. It is naturalised throughout Australia, is self pollinated and has the ability to produce numerous wind borne seeds.

The aim of this work was to investigate the existence of acetolactate synthase-inhibiting (ALS-inhibiting) herbicide resistance genes in *S. oleraceus* and the pattern of genotypes carrying these genes in a single field.

Seed from 37 *S. oleraceus* plants was collected from a field at Roseworthy, South Australia with plant locations recorded using a Global Positioning System (GPS). DNA was extracted from the seedlings grown from the seed and used for Amplified Fragment Length Polymorphism (AFLP) analysis to assess the genetic relationships between individuals. The seedlings were treated with chlorsulfuron, an ALS-inhibiting herbicide, revealing 15 resistant plants and 22 susceptible to the herbicide. The AFLP analysis indicated the 15 resistant plants were located in four separate genetic groups. This suggests evolution of resistance independently in several genotypes, which may or may not have occurred *in situ*.

Keywords Sowthistle, *Sonchus oleraceus*, gene flow, AFLP analysis, ALS-inhibiting herbicide resistance.

INTRODUCTION

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 increase in frequency with frequent use of herbicides of the same mode of action. It is also possible that resistance alleles may enter populations from outside. Such gene flow may be a particular issue where resistance alleles are infrequent or absent from a population and may greatly increase the speed of resistance selection.

Herbicide resistance gene flow may occur through pollen or by seed. For example, herbicide tolerant

canola has been shown to pollinate susceptible individuals at 2.6 km from the source (Rieger *et al.* 2002). Where a plant species is self-pollinating seed movement is the only avenue for resistance alleles to enter a population. Seed dispersal can also be important and the rapid dispersal of wind blown seed over large distances has been reported in *Lactuca serriola* L. (Lu 2005).

Sonchus oleraceus is a wind dispersed weed having achenes with a pappus enhancing dispersal, but have also been reported to have been dispersed by water and birds (Holm *et al.* 1977, Hutchinson *et al.* 1984, Anderson 1991). *S. oleraceus* seed has little dormancy and readily germinates (Widderick 2002) so the gene pool changes rapidly. Herbicides are effective in controlling this weed, but in 1991 the first *S. oleraceus* resistant to ALS-inhibiting herbicides was recorded in Australia (Boutsalis and Powles 1995).

Sonchus oleraceus is self pollinated and any gene flow must occur through seed movement. Through knowledge of the major mechanisms of gene flow, 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 kill the plants prior to flowering, reducing the possibility of gene flow. This may not be the only successful method of controlling gene flow and understanding the mechanisms of gene flow can assist in selecting the best weed control management methods.

The aim of this work was to use AFLPs to elucidate the movement of resistant genotypes of *S. oleraceus* in a localised region of South Australia and provide an opportunity for improving current management practices.

MATERIALS AND METHODS

Sonchus oleraceus plants were sampled from a 22 ha field located at Roseworthy Agricultural College 50 km north of Adelaide, South Australia (34°32'50"S,

138°41'25"E). One to five capitulum seed heads per plant were collected from 37 individuals in the field in November 2006. Each plant's location was recorded using a GPS.

Seedlings were germinated from the collected seed. Leaf material was collected from one seedling from each of the 37 seed samples and used for DNA extraction. Seedlings were then treated with 15 g a.i. ha⁻¹ 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⁻¹ at a pressure of 240 kPa with a boom speed of 1 m s⁻¹. Thirty days after treatment plants were scored as alive (resistant) or dead (susceptible).

DNA was extracted using a 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 was viewed with GeneMapper® software for the presence or absence of peaks which were analysed using PopGene® software to determine genetic relationships.



Figure 1. Aerial photograph of the field at Roseworthy, South Australia (Google Earth 2007) showing the locations of the 15 resistant plants with different genotypes indicated (R1, R2, R3 and R4) and the 22 susceptible plants. (S1 to S22).

RESULTS

Of the 37 *S. oleraceus* individuals tested, 15 were resistant to chlorsulfuron and 22 were susceptible. AFLP analysis indicated the 15 resistant plants distributed into 4 separate genetic groups in the field (Figure 1). These groups tended to cluster within the field, but some were distributed more widely.

DISCUSSION

The detection of four separate groups of resistant genotypes (R1, R2, R3 and R4) in the field suggests independent evolution of resistance has occurred in several individuals. This could have occurred *in situ*, but may also have resulted from wind blown seed successfully colonising patches in the field. The location of the resistant individuals within the field suggests wind borne seed has played a role in the spread of chlorsulfuron resistant *S. oleraceus*.

Genetic relationships over large areas were investigated in *L. serriola* (Lu *et al.* 2007). This study found that 25 *L. serriola* plants collected over less than 5 km were identical and earlier collections over a larger area had greater genetic variance. Seed movement as far as 43 km was also reported. This species has seed similar to *S. oleraceus* and is also self-pollinated. Thus, dispersal patterns from *S. oleraceus* seed could be expected to be similar to that of *L. serriola*.

Further work needs to be conducted to determine if the mutations occurred within the field or if incoming seed gave rise to the resistant patches. Additional samples of *S. oleraceus* have been collected from around this field. The genotypes and spatial distribution obtained from these samples may elucidate the driving forces behind the evolution of resistance in the field by revealing the extent of gene flow in a localised area. Given that *S. oleraceus* is a self-pollinated species, 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.

ACKNOWLEDGMENTS

Thanks to Dr John Stephen and staff from the Australian Genome Research Facility, Plant Genomics Centre, University of Adelaide, South Australia and special thanks to Nicole Burt for final analysis of AFLP PCR product and assistance with GeneMapper software.

REFERENCES

- Boutsalis, P. and Powles, S.B. (1995). Resistance of dicotyledon weeds to acetolactate synthase ALS-inhibiting herbicides in Australia. *Weed Research* 35, 149-55.
- Anderson, S. (1991). Geographical variation and genetic analysis of leaf shape in *Crepis tectorum* (Asteraceae). *Plant Systematics and Evolution* 178, 247-8.
- Google Earth. (2007). Viewed 22 October 2007. <http://www.google.com>
- Holm, L.G., Plucknett, D.L., Pancho, J.V. and Herberger, J.P. (1977). The world's worst weeds – distribution and biology. pp. 436-39 (The University Press of Hawaii, Honolulu).
- Hutchinson, I., Colosi, J. and Lewin, R.A. (1984). The biology of Canadian weeds. 63. *Sonchus asper* (L.) Hill and *S. oleraceus* L. *Canadian Journal of Plant Science* 64, 731-44.
- Lu, Y.Q., Baker, J. and Preston, C. (2007). The spread of resistance to acetolactate synthase inhibiting herbicides in wind borne, self-pollinated weed species, *Lactuca serriola* L. *Theoretical Applied Genetics* 115, 443-50.
- Lu, Y.Q. (2005). The spread of herbicide resistance in *Lactuca serriola* at a landscape scale. MSc thesis, Discipline of Plant and Pest Sciences, University of Adelaide, South Australia.
- Preston, C. and Powles, S.B. (2002). Evolution of herbicide resistance in weeds: initial frequency of target site-based resistance to acetolactate synthase-inhibiting herbicides in *Lolium rigidum*. *Heredity* 88, 8-13.
- Rieger, M.A., Lamond, M., Preston, C., Powles, S.B. and Roush, R.T. (2002). Pollen mediated movement of herbicide resistance between commercial canola fields. *Science* 296, 2386-8.
- Vos, P., Hogers, R., Bleeker, M., Reijmans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. (1995). AFLP: a technique for DNA fingerprinting. *Nucleic Acids Research* 23, 4407-14.
- Walker, S.R., Widderick, M.J. and Galea, K. (2005). Stopping herbicide resistance in southern Queensland. Queensland Government. Department of Primary Industries and Fisheries, Brisbane, Queensland.
- Widderick, M.J. (2002). Ecology and management of the weed common sowthistle (*Sonchus oleraceus* L.) PhD thesis, University of New England, Armidale, NSW.