DISPERSAL AND GENETIC VARIABILITY OF *Sonchus oleraceus* L. IN RELATION TO ITS RESISTANCE TO ALS-INHIBITING HERBICIDES

Robin. S. St John-Sweeting
R.D.A., Grad.Dip.Ag., Master of Applied Science (Agriculture)

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DISPERSAL AND GENETIC VARIABILITY OF *Sonchus oleraceus* L. IN RELATION TO ITS RESISTANCE TO ALS-INHIBITING HERBICIDES

Sonchus oleraceus **L. (common sowthistle)**
An annual weed with potential to distribute numerous wind borne seeds.

" To win the secrets of a weed's plain heart."

James Russell Lowell (Sonnet XXV).

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Abstract

The work described in this thesis investigates the existence and level of acetolactate synthase (ALS)-inhibiting herbicide resistance in *Sonchus oleraceus* in Australia. It further discusses the sensitivity of different *S. oleraceus* populations to different dose rate treatments of the ALS-inhibiting herbicide, chlorsulfuron. Thirdly the movement or not of the resistance gene between *S. oleraceus* plants. Gene movement is investigated in light of *S. oleraceus* being self pollinated and possessing a wind dispersed seed. Finally using molecular tools the genetic diversity and seed movement in *S. oleraceus* is investigated.

Although much is known about the evolution of plant based genetic resistance to herbicides there is less known as to the specific resistance gene movement in differing weed species.

The first approach undertaken in this study was to collect a broad spectrum of *S. oleraceus* seed from a number of Australian states and test the progeny from this seed for resistance to chlorsulfuron. Subsequent to this DNA extractions were made from *S. oleraceus* plant material for use in AFLP and sequencing techniques.

The results of this study indicate that ALS-inhibiting herbicide resistance to chlorsulfuron in *S. oleraceus* is now widespread in Australia. The movement of the resistance gene within populations is low (<4%), however, population dendrograms indicate seed has been dispersed across large distances in Australia facilitating the movement of the resistance gene. In addition sequence analysis indicates numerous independent mutation events. With the identification of previously unknown levels of resistance in Australia and gene movement knowledge, extension of improved management practises is possible.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Robin St. John-Sweeting and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text.

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Robin St. John-Sweeting

22 September, 2011

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Poster and one page paper at 15th Australian Weeds Conference, Adelaide.

St. John-Sweeting, R.S., Preston, C., Baker, J., Walker, S. and Widderick, M. (2006) Herbicide resistant sowthistle (*Sonchus oleraceus* L.) in South Australia, northern New South Wales and Queensland. Proceedings of the 15th Australian Weed Conference, eds. C. Preston, J.H. Watts and N.D. Crossman, pp. 3-13. (Weed Society of South Australia, Adelaide).

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Poster

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Abbreviations

3'	three prime end of nucleic acid
2,4-D	2,4-dichlorophenoxy acetic acid
5'	five prime end of nucleic acid
ACPFG	Australian Centre for Plant Functional Genomics
AFLP	amplified fragment length polymorphism
AGRF	Australian Genome Research Facility
ALS	acetolactate synthase
bp	base pair (s)
Chlorsulfuron	2-chloro-N-{{(4-methoxy-6-methyl-1,3,5-triazin-2 yl)amino}carbonyl}benzenesulfonamide;
CTAB	cetylmethylammonium bromide
DNA	deoxyribonucleic acid
dNTPs	2'-deoxynucleotide triphosphatases
EDTA	ethylenediamine *tetra* acetic acid
EtBr	ethidium bromide
GPS	global positioning system
ISSR	inter-simple sequence repeat
kb	kilobase; kilobase pairs
metsulfuron-methyl	methyl-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino]carbonyl]aminosulfonyl]benzoate
MQ Water	18mΩ water (Milli-Q ultrapure water system)
MW	molecular weight
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism
RL `	restriction ligation
RO	reverse osmosis
TAE	tris-acetate/EDTA buffer
Tris	tris(hydroxymethyl)aminomethane

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Glossary of Terms

Accession — A new member of a plant collection.

Biotype — A group of individuals with the same genotype.

Cluster — A geographically bounded concentration of similarly related plants.

Cross Pollination — Pollen is exchanged between different flowers from the same or different plants. Also known as syngamy or allogamous species.

Flushes — Natural revegetation of plants in the field.

Mutation — A heritable change that occurs in genetic material. It may lead to a different number of repeats of a certain sequence or a change in one of the bases in a sequence.

Mutation rate — Frequency with which random mutations occur.

Pappus — Small hairs borne at the tip of a seed.

Phenotypic — Observed quality of an organism such as morphology, development or behaviour based on its genetic and environmental situation.

Pollination — Transfer of pollen grains containing the male gametes to the plant carpel of female flowering parts containing the ovule which houses the gametes.

Population — A number of plants of a particular genotype. An assemblage of organisms living in a given area.

Self-Pollination — Pollination that occurs within a single flower usually before it opens. Also known as autogamy or occurring in an autogamous species.

CHAPTER 1

1 GENERAL INTRODUCTION

1.1 BACKGROUND

Sonchus oleraceus L. (common sowthistle) is a weed of major importance of cropping areas in the northern grain region of Australia (NGRA). The species is a weed prominent in fallows, using moisture and interfering with crop harvest. It is also a weed of lesser importance in cropping systems in the southern and western grain growing regions. Competitive crops will greatly reduce *S. oleraceus* weed biomass and potential weed seed production and, when effectively managed, this weed has minimal effect on the farming enterprise (Widderick 2002). However, in the last two decades, numerous populations of *S. oleraceus* in southern Queensland have evolved resistance to acetolactate synthase (ALS)-inhibiting herbicides, such as chlorsulfuron and metsulfuron-methyl. There is a risk of more weeds evolving resistance to ALS-inhibiting herbicides in winter rotations where these herbicides are used intensively (Walker *et al.,* 2005b). The evolution of ALS-inhibiting herbicide resistance complicates management of this weed. Herbicide resistance threatens the efficiency and profitability of agricultural enterprises both nationally and worldwide. General principles and strategies have now been developed and applied to avoid the increasing development of herbicide resistance in weeds.

The Weed Science Society of America, after considering the influence of weed resistance in Australia, emphasised that it is imperative that research continues into weed resistance and management (Hall *et al.,* 2000). Corbett and Tardif (2006); Tranel and Wright (2002) highlight that ALS-inhibiting herbicides have been used worldwide due to their high activity, selectivity and wide spectrum. Due to the intensive and persistent use of these herbicides over 100 weed species have been reported to have evolved resistance to ALS-inhibitors (Heap 2010). Little is known about the spread of ALS resistance by wind dispersed seed and an

understanding of the evolution and dispersal of this resistance is likely to be helpful not only in managing *S. oleraceus*, but also managing resistance to other herbicides in self pollinated wind dispersed weeds.

1.2 BIOLOGY AND ECOLOGY

S. oleraceus is an erect annual winter herb that reproduces only by seed (Hutchinson *et al*. 1984). It is common in disturbed habitats and waste places and usually grows to a height of 400 to 700mm, but has been known to reach a height of 1500mm. Its tap root is unbranched and stems are hollow with alternate pinnately-lobed leaves with small auricles. The inflorescence is irregularly cymose-umbellate carrying a number of capitulum, each containing approximately 150 bisexual yellow florets (Hutchinson *et al*., 1984). The flower buds increase in size until maturity at which time the bud opens exposing the yellow composite florets. Each floret has one ovule with the stigma and style extending upward through a tube containing grains of pollen, at which time self pollination occurs (Lewin 1975). The flower opens only one morning then closes and stays closed for approximately one week while the seed achenes mature. After maturation, the seed head opens in the afternoon and the seed with pappus attached is dispersed by the wind. However, Widderick pers. comm. (2008) reports seed dispersal in the morning in Queensland when the temperatures are high enough. The seed has little to no innate dormancy and has an intermittent and prolonged emergence period (Widderick 2002).

1.3 AIMS AND SCOPE OF THIS THESIS

1.3.1 INTRODUCTION

This study will focus on genetic diversity, gene flow and spatial distribution of ALS-inhibiting herbicide resistance in *S. oleraceus*. With the aid of molecular genetic tools, this project will

assess the type and level of genetic variation within and between *S. oleraceus* populations with an emphasis on herbicide resistant populations.

Agricultural production is reduced by competition with weeds. Agriculturalists have used numerous weed management control methods to reduce the competitive effect of weeds, such as herbicides (Gressel and Segal 1982; Hashem and Bowran 2001). The use of herbicides has resulted in large increases in agricultural production (Mathews 1994). These increases are threatened with the evolution of plant based genetic resistance to these herbicides. Interest in assessing this evolution in weeds has focused on genetic mutations, gene flow and the selection of resistance genes in plant populations (Messeguer *et al*., 2001; Knispel *et al.,* 2008). Gene flow can have an important role in disseminating resistance and can affect the level of resistance and the consequent impact on crop production (Ashigh *et al*., 2008). The first recorded biotype of *S. oleraceus* resistant to ALS inhibiting herbicides in Australia was found at Goondiwindi, Queensland in 1991 (Boutsalis and Powles 1995a). This poses the question of where else resistance occurs in Australia. In addition, it is unknown how or whether resistance is moving over the landscape. Also it is not known how much genetic variation is present in Australian populations of *S. oleraceus.* The project aims to assess the pattern of ALS-resistance gene movement and genetic diversity of *S. oleraceus,* with particular emphasis on plants with resistance to herbicides. Literature relating to the biology and ecology of *Sonchus oleraceus*, herbicide resistance, population genetics and the evolution of herbicide resistance and molecular genetics will be thoroughly reviewed in Chapter 2. The general methods, including seed collection are covered in Chapter 3.

1.3.2 HERBICIDE RESISTANCE IN AUSTRALIAN *S. oleraceus*

Chapter 4 aims to assess the distribution of resistance alleles from *S. oleraceus* local populations in Queensland, New South Wales, South Australia, Western Australia, Victoria

and Tasmania. Seed collections from 1991 and 1998 to 2001 were used along with new seed collections from 2005 to 2008. DNA was extracted and preliminary investigations conducted prior to selection of the most suitable methodology. Seed was propagated and treated with the Group B sulfonylurea herbicide chlorsulfuron to determine whether resistance existed. A dose response experiment with chlorsulfuron was conducted to determine the sensitivity of a number of different *S. oleraceus* populations to different dose rate treatments.

1.3.3 POLLEN MOVEMENT IN *S. oleraceus*

Chapter 5 investigates whether gene flow can occur by pollen movement between plants in *S. oleraceus*. Individual resistant and susceptible plants were propagated and pollen movement experiments conducted. Plants were placed outside and allowed to flower and set seed naturally. The seed from the susceptible plants was collected and tested for herbicide resistance to assess whether resistance genes had moved to the susceptible plants in pollen.

1.3.4 GENETIC DIVERSITY & GENE MOVEMENT IN *S. oleraceus*

Chapters 6 and 7 investigate the genetic diversity of *S. oleraceus* with particular emphasis on plants with resistance to herbicides. Genomic DNA was extracted from collection samples and analysed using fluorescent amplified fragment length polymorphisms. Amplified bands or peaks were scored as present or absent and genetic relationships tested. Evidence of widespread seed movement or multiple *de novo* mutations events can be drawn from the finding of the same genotype at multiple locations.

The findings of the research will be brought together and are discussed in Chapter 8 which will also include implications of the research and future research priorities in this study area.

CHAPTER 2

2 LITERATURE REVIEW

2.1 INTRODUCTION

This literature review briefly covers the biology of members of the family Asteraceae and reviews relevant literature relating to *Sonchus oleraceus* L. (common sowthistle). It introduces the Australian agricultural environment as a habitat of *S. oleraceus*. Relevant aspects of weed science are covered; with an emphasis on the evolution of resistance to herbicides inhibiting acetolactate synthase (ALS) also called acetohydroxyacid synthase (AHAS). Within this group of herbicides the chemical family of sulfonylureas are a focus. The evolutionary development, genetics and molecular genetics of *S. oleraceus* are covered.

S. oleraceus is a weed of bare ground (Walker pers. comm. 2005). Martin *et al*., (1988) observed *S. oleraceus* as a weed of fallows rather than competing with crops. *S. oleraceus* is reported as a host to a number of pests and diseases of crops. Included among these pests and diseases are aphids, some species of which are vectors of the disease Lettuce Necrotic Yellow Virus (O'Loughlin and Chambers 1969), fungal pathogens, other insects and nematodes. Therefore, the weed's main impacts are as a user of resources in fallow, as a host for pests and diseases, a contaminant at harvest and as direct competition for resources in crops.

The introduction of chemicals for the control of weeds in the 1900s have included sodium chlorate (1910), 2,4-D (1942), diuron, amitrole, simazine, paraquat and numerous others in the 1950s (Anonymous 2004). Others chemicals including bromoxynil, chlorthal-dimethyl and oryzalin were developed in the 1960s and glyphosate and oxyfluorfen to name a few in the 1970s. The first sulfonylurea herbicide chlorsulfuron (Glean®) was released in 1979. Chlorsulfuron was followed by sulfometuron-methyl (Oust®) in 1980, Metsulfuron-methyl (Ally®) in 1985 and triasulfuron (Logran®) in 1985 (Anonymous 2004). Others sulfonylurea

herbicidess have been released in the late 1980s and 1990s and are all ALS-inhibitors. Since the 1950s crop production has become increasingly dependant on the use of herbicides to control weeds with this benefit has been diminished as weeds have evolved resistance to the herbicides.

Throughout the world weed species are accumulating resistance mechanisms, evolving multiple resistances across many herbicides posing a great challenge to herbicide sustainability in world agriculture (Powles and Yu 2010). Herbicide resistance has evolved as a result of selection for individuals within weed populations that survive herbicide treatment (Tranel and Wright 2002; Holt *et al*., 1993; Tucker and Powles 1988). The first weed biotype resistant to ALS-inhibiting herbicides (*Lactuca serriola* L.) was identified in 1987 (Mallory-Smith *et al*., 1990a). Although usually present at very low frequency, weed populations often contain individuals that are naturally resistant to ALS-inhibiting herbicides and which increase in numbers over generations as a constant selection pressure is applied (Preston and Powles 2002). It is also possible that a novel mutation could be introduced from outside the population, if none were present within a population. For *S. oleraceus*, like other self-pollinated weeds, resistant alleles are dispersed through seeds. This dispersal can be by wind, water, animal or mechanical means and once dispersed, establishment and reproduction can occur as the seed establishes new populations of resistant plants. The level of success of these dispersed populations rests with the fitness of the immigrant individuals compared to existing individuals. The emergence of herbicide resistance will depend on the fitness of individuals to survive the application of herbicides.

Factors that influence herbicide resistance evolution include the herbicide use pattern, the number of plants treated, the mode of inheritance of the gene endowing resistance and the initial frequency of the herbicide-resistant individuals (Powles *et al*., 1997). Resistant

populations have been found in numerous geographic locations at various distances apart. Whether these populations originated from a single mutation conferring resistance of multiple mutation events can only be determined by further analysis of their genetic relationship (Ashigh and Tardif 2007).

The first recorded biotype of *S. oleraceus* resistant to ALS-inhibiting herbicides in Australia was found at Goondiwindi, Queensland in 1991 (Boutsalis and Powles 1995a). Adkins *et al*., (1997) reported *S. oleraceus* as one of 15 weed species resistant to the recommended rate of chlorsulfuron (15g a.i. ha^{-1}) in a survey of weed resistance in the north-east grain region of Australia.

The direct economic and environmental impact to Australian ecosystems caused by *S. oleraceus* in the late 1990s was described as being relatively low (Widderick 2002). However, this is likely to increase with selection for an increased frequency of resistant plant biotypes in populations. Recent surveys conducted in 2001 of cotton growers in southern Queensland and northern NSW found *S. oleraceus* to be a common problem weed (Walker *et al.,* 2005a; Alemseged *et al*., 2001).

Gilbey (1974) outlined the relationship between the density of the broad leafed weed *Emex australis* and found that 10 plants per m^2 reduced wheat yields by 10% and 120 plants per m^2 reduced yields by 50%. Since *S. oleraceus* is also a broad leaf weed and is likely to have similar competitive effects to *E. australis,* it could be assumed that without appropriate management strategies to control populations of *S. oleraceus*, their increased presence will reduce crop yields. Strategies to prevent, or minimise, the risk of resistance are now in place (Walker *et al.,* 2005b): However, farmers and land managers must be convinced to adopt such strategies based on a sound understanding of the weeds ecology.

This project will investigate gene flow among *S. oleraceus* populations and the implications that movement of herbicide resistance between *S. oleraceus* populations will have on resistance management. It will investigate the genetic diversity and the dispersal and spread of *S. oleraceus* on a landscape scale and suggest development of resistance management programs based on the results.

2.1.1 *Sonchus oleraceus* IN THE AGRICULTURAL ENVIRONMENT

The Australian agricultural environment is mainly situated in the rain-fed areas within 600 km of the coast as most of the centre of Australia is arid or semi arid. The grain growing regions are divided into the western, southern and northern grain regions of Australia (Figure 2.1).

The climate in much of the southern and western grain regions is similar to that of countries around the Mediterranean basin, parts of Chile and Argentina in South America, South Africa, parts of south western Asia and the western United States of America. Winters are mild and humid and summers are hot and dry. The southern grain region of Australia (SGRA) and western grain region of Australia (WGRA) have a temperate climate, while the northern grain region of Australia (NGRA) ranges from temperate to sub tropical in New South Wales through to sub tropical in southern Queensland. *S. oleraceus* has been recorded in all of these regions. Widderick (2002) found that *S. oleraceus* is a common weed throughout the NGRA and it infests all important crops and fallows using moisture and interfering with harvest. Although geographically widespread, *S. oleraceus* is not very competitive and growing a competitive crop greatly reduces the weed's biomass and potential seed production (Widderick 2002). Common sowthistle is currently the most prevalent weed of cropping in southern Queensland where it has increased in importance in the last few decades as farming systems have moved towards reduced or zero tillage (Widderick pers. comm. 2009).

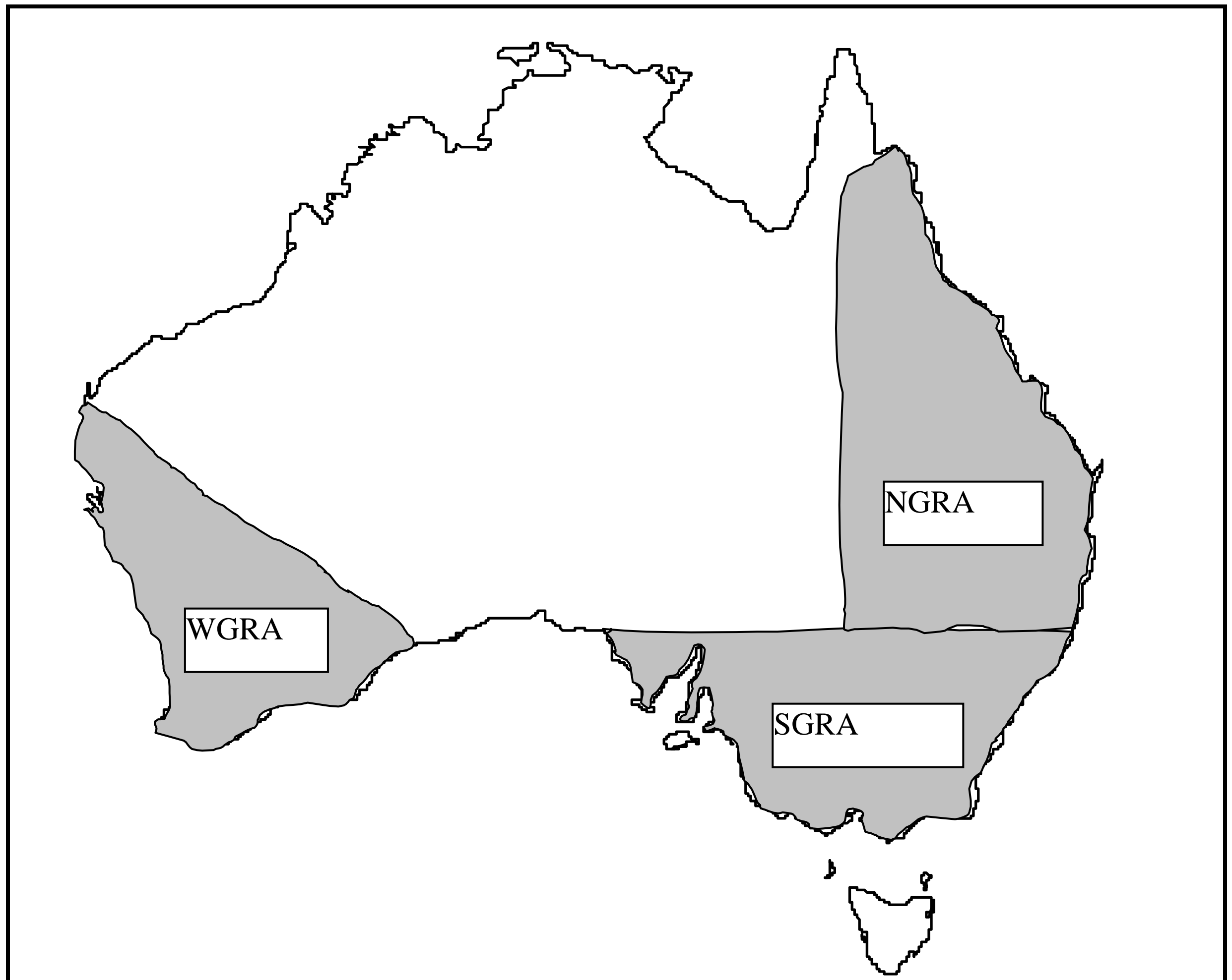

Figure 2.1 Location of the western, southern and northern grain regions of Australia. (WGRA = western grain region of Australia, SGRA = southern grain region of Australia and NGRA = northern grain region of Australia). Note: Locations are generalised areas.

The surface (0-1 cm) seed bank is depleted by germination following rain and via other means, such as predation or decay. In contrast, up to 12% of seeds buried below a depth of 2 cm persisted for at least 30 months (Widderick 2002).

2.2 TAXONOMY, BIOLOGY AND ECOLOGY

2.2.1 THE FAMILY ASTERACEAE

The Asteraceae is the largest angiosperm family with 1100 genera worldwide and 200 genera native to Australia (Black 1986). Judd *et al*., (1999) lists the characteristics that plants in the family Asteraceae share: an inflorescence with a capitulum flower head, anthers with stamens fused together at their edges forming a tube, ovary with basal arrangement of the ovules, one ovary per ovule, an achene fruit with a tuft of hair (pappus).

2.2.2 THE GENUS *Sonchus*

2.2.2.1 *Sonchus* in the world

There are 55 species of *Sonchus* worldwide (Boulos 1972). Boulos (1972) had linked the genus *Sonchus* with the genus *Embergeria*. This caused some controversy, but now it is generally regarded separately as *Sonchus*.

2.2.2.2 *Sonchus* in Australia

There are 2 native and 4 naturalised species of *Sonchus* (with 2 *asper* subspecies) in Australia (Table 2.1). This study will focus on *S. oleraceus*, a member of the family Asteraceae.

Table 2.1. *Sonchus* species in Australia (Boulos 1972).

NOTE:
This table is included on page 10
of the print copy of the thesis held in
the University of Adelaide Library.

2.2.2.3 Geographic distribution of *S.oleraceus*

Origin and world distribution of S.oleraceus

The already wide geographical distribution of *S. oleraceus* globally makes determining the geographic origin difficult (Mitich 1998). Guertin and Halvorsen (2003) report that *S. oleraceus* is a native to Africa (Mediterranean region), temperate Asia, tropical Asia, and Europe. Lewin (1948) described *S. oleraceus* as a weed that had followed the spread of civilization to the temperate and sub-tropical zones of both hemispheres and high altitudes in the tropics to their present distribution worldwide (Figure 2.2).

The current world distribution of *S. oleraceus* is very broad and covers areas of northern Europe and Canada through the tropics to South America and South Africa (Holm *et al*., 1977), United States of America (Peschken 1982), Canada (Hutchinson *et al*., 1984) and England (Mitich 1998).

Figure 2.2 Global distribution of Sonchus oleraceus L (Holm *et al*. 1977) (Australian Virtual Herbarium 2009).

Australian distribution of S. oleraceus

S. oleraceus is widespread throughout Australia (Auld and Medd 1987; Australian Virtual Herbarium 2009). In Queensland, *S. oleraceus* is found in the regions of Moreton, eastern Darling Downs, western Darling Downs, Maranoa and central Queensland. In New South Wales, it is found in the Central-West Slopes and Plains, North-West Plains and North-West

Slopes. It is also widespread throughout South Australia, Victoria, Western Australia, Tasmania and the Northern Territory (Figure 2.3).

NOTE:
This figure is included on page 12 of the print copy of the thesis held in the University of Adelaide Library.

Figure 2.3 Australian distribution of *Sonchus oleraceus* L. derived from existing physical herbarium records (Australian Virtual Herbarium 2009).

2.2.2.4 Morphology and variation

S. oleraceus stems are commonly 20 to 150 cm in height, hollow, septate at the nodes and more or less pentagonal in section by reason of the decurrent midribs in the leaves (Alex *et al.,* 1980). The leaves are not spinous or glossy and can vary in shape, even within the same plant. The plant is glabrous except for a caducous tomentum around the young leaf and flower buds (Hutchinson *et al*., 1984). Clapham *et al*., (1987) point out a large amount of leaf shape variation in the species. *S. oleraceus* plants from the northern grain region of Australia are morphologically diverse varying in leaf colour, leaf serration, rosette form, height,

fecundity and time of flowering (Widderick 2002). Andersson (1991) reported that self-pollinating weed species, such as *S. oleraceus*, are more likely to form distinct biotypes compared with species with a tendency to out-cross. A survey conducted in the northern grain region of Australia highlighted that: *S. oleraceus* is widespread and prevalent; that it is probable that biotypes of *S. oleraceus* exist; and that the weed's ability to adapt may partly be responsible for its prevalence (Widderick 2002). A high degree of morphological variation (Widderick 2002) may indicate a high level of cross pollination and genetic variation; however, this is perplexing as the plant is regarded as a self pollinator (Barber 1941). Whatever the reason for these morphological differences the fact is that self pollination will perpetuate them.

2.2.2.5 Habitat and ecology

In general, climate is the main limiting factor to the potential distribution of a plant species. The climatic limitations of *S. oleraceus* are not fully understood; however, the distribution of *S. oleraceus* would indicate a broad tolerance of climatic variation (Hutchinson *et al*. 1984). In Australia, *S. oleraceus* is found in areas with climate ranging from arid to tropical, including both dry and wet summer areas (Widderick 2002). In Canada, the distribution of annual *Sonchus* spp. is within a range of precipitation from 300 to 3000mm (Guertin and Halvorson 2003). *S. oleraceus* prefers well drained, slightly acid to alkaline soils and is tolerant of saline soils (Lewin 1948). Hutchinson *et al.,* (1984) reports *S. oleraceus* likes nutrient-rich, not too dry, nitrogen containing soils with textures that are loamy sands or stony.

2.2.2.6 Plant development

There is no vegetative reproduction in *Sonchus* and unfavourable periods of growth are mitigated by a large seed bank that can persist for up to 30 months (Lewin 1948, Widderick 2002). In the northern hemisphere specimens that have survived the winter commence

flowering about the middle of March to the end of April. Achenes are produced abundantly between May and November and in suitable conditions will germinate (Lewin 1948). Mature achenes are produced about a week after flowering. Capitular bracts open when the achenes are mature and then the pappus-borne achenes are dispersed by the wind (Hutchinson *et al*., 1984). In the southern hemisphere, specimens flower from October through the summer and into autumn depending on the time of germination, which can be variable (Widderick 2002).

2.2.2.7 Karyology

The chromosome number of *S. oleraceus* is 2n=32. Cooper and Mahony (1935) described it as a tetraploid and that there is some evidence of secondary association of bivalents on the metaphase plate in the first division of the pollen mother cells. Stebbins *et al*., (1953) described *S. oleraceus* as an allotetraploid or amphidiploid with a chromosome number of 32 with 18 chromosomes coming from *S. asper* and 14 chromosomes from *S. tenerrimus* L. It is interesting to note the findings of Stebbins *et al.,* (1953) indicate that the two different species of *Sonchus* can cross, producing fertile progeny when *S. oleraceus* is the female. However, data compiled by Hsieh *et al*., (1972) indicated that *S. oleraceus* is an autotetraploid. As a point of interest, Marchal (1920) reported a diploid (2n=16) variant.

The chromosome number is important in understanding the genetic nature of the plant and the position of resistant alleles on the chromosome as they are mapped. There are also implications for genetic diversity and mutation. Chromosomal variation is widespread in plants and animals. It often contributes to the genetic barriers preventing gene flow between species and hence its role in species diversification has been heavily debated (White, 1978; King, 1993; Rieseberg, 2001). Our understanding of these phenomena has progressed considerably recently as a result of theoretical research on hybridization and genome duplication and technical advances in Polymerase Chain Reaction (PCR).

2.2.2.8 Reproduction

Floral biology and flowering

Unlike the glabrous stems and leaves, the flower buds have caducous tomentum and glandular hairs sometimes present on the branches and involucres of the inflorescence, which is irregularly cymose-umbellate (Lewin 1948). The involucres are about 1.5cm long and the span of the capitulum when open is 2 to 2.5cm. The florets are ligulate and yellow. The pappus is white and silky (Lewin 1948). Percival (1955) observed the duration of pollen presentation to be between 7 am and 12 noon, with a peak period of 8 to 9 am. On 84% of the days, pollen was present during this peak period. In addition, from observations of 86 plant species, it was noted that *S. oleraceus* produced the least amount of pollen, but that nectar was available and that pollen collection did not occur by bees. Percival (1955) also states that anthesis in *S. oleraceus* occurs at the highest relative humidity level of 95%, with free anthesis at 15 to 22.8^{o}C and that rain limited flower opening. The capitulum contains some 200 florets each of which consist of an ovary, pappus and anther-collar tube through which the stigma and style emerge.

Pollination

Pollination is an important factor in the flow of genes from one plant to another. Barber (1941) states that *S. oleraceus* is self fertile and attempts to cross artificially were unsuccessful, although Stebbins *et al*., (1953) indicated crossing was possible when *S. oleraceus* was the maternal donor. Lewin (1975) reported that true hybrids involving *S. oleraceus* were rare and that it was unlikely that many of the reported "hybrids" between *S. oleraceus* and *S. asper* were of mixed descent. Barber (1941) describes an undoubted hybrid, which arose spontaneously in cultivation in which the chromosome number was 25 (*S. oleraceus,* 2n=32: *S. asper,* 2n=18), but the plant was sterile both in pollen and in the ovules.

There are some contradictory findings on the success of outcrossing, although the reasons for these differences are not clearly understood. Fitzpatrick *et al*., (2003) found that the level of gene flow in the cross pollinated plant, *Medicago sativa* (lucerne/alfalfa) to be 1.39% at 500 ft separation, 0.28% at 900 feet, 0.08% at 1500 feet and 0.0% at 2000 feet. Their work further indicated that with separation distances of greater than 900 feet that this isolation standard is sufficient to produce seed with 99.7% varietal purity. So, it is clear that distance between donor and source can affect the level of gene flow. However, for the purpose of this study, and consistent with the findings of Pupilli *et al*., (2004) and Lu *et al*., (2008), it will be assumed that *S. oleraceus* is self pollinated and produces seed that is genetically identical to the mother plant.

Seed production, dispersal and viability

The tall stems of sowthistles aid the seed dispersal process by elevation above the crop canopy (Sheldon and Burrows 1973). *S. oleraceus* achenes are distributed by wind, with the pappus enhancing dispersal, but have also been reported to have been dispersed by water and birds (Andersson 1991; Holm *et al.,* 1977; Hutchinson *et al.,* 1984). Methods of reducing the spread of wind dispersed seeds may be impractical on a large scale. However, Davies and Sheley (2007) suggest a conceptual framework for preventing spatial dispersal of invasive plants by identifying vectors of dispersion and management strategies. This recognises that preventing invasive plants from infesting new areas is more cost-effective and efficient than trying to restore the system after it is infested.

Salisbury (1964) reports that seed may germinate after ingestion and excretion by birds and mammals. For example 27% of the achenes of *S. asper* fed to a cow germinated in the manure (Dorph-Peterson 1924). This demonstrates the hardy seeds, particularly since the cow is a ruminant. Zollinger and Parker (1999) report *Sonchus* achenes found tangled in the wool

or hair of animals. Guertin and Halvorson (2003) report from Holm *et al*., (1977) that *Sonchus* seeds have been collected by aircraft on a screen at 600m. This shows the potential for achenes to blow widely over the landscape and hitchhike on animals.

Shields *et al*., (2006), using remote controlled model aircraft, collected *Conyza candensis* (horseweed) seeds, a member of the Asteraceae also with wind-borne seed, up to 140m above the ground and suggested that seeds can easily be dispersed more than 500km in a single dispersal event. Lebeda *et al.,* (2004) found that of populations of *Lactuca* spp. collected in Europe, 59% originated in Europe, 37% in Asia and 2% from Africa and 2% from America. This highlights the mobility of wind dispersed seed or human mediated movement.

Harper (1977) reported that in most plant species the population of dormant seed in the ground is often much greater than the above ground growing population. *S. oleraceus* seeds lack innate dormancy and readily germinate over a wide range of temperatures (Widderick 2002). Weeds that emerge with a crop can do far more competitive damage than weeds that emerge later in the crops' development (Sanyal *et al*., 2008). Although *S. oleraceus* is not very competitive, with an increased frequency of herbicide resistant plants would be expected to increase the potential for adverse competition.

2.2.2.9 Parasites and predators

S. oleraceus is host to a number of insects including aphids, flies, leaf miners and seed borers (Hutchinson *et al.,* 1984). Berube (1978) reported two flies (*Tephritis dilacerate* and *T. formosa*) that occasionally lay eggs into the flower buds of *S. oleraceus* forming galls. *S. oleraceus* is host to a number of nematodes that reduce the vigour of the plant (Hutchinson *et al.,* 1984). An eriophyid mite which causes leaf edge curl and swelling has been found in Western Australia (McCarren pers. comm. 2007). This knowledge is of value in beginning the process of the selection of any biological control agent for the control of *S. oleraceus* in Australia. However, there is little evidence currently that specific predators are important in controlling populations of *S. oleraceus*.

2.2.2.10 Control measures

Response to herbicides and other chemicals

S. oleraceus is susceptible to a wide range of pre-emergence and foliar herbicides from a number of herbicide groups (Table 2.2). This suggests that developing a strategy to prevent the evolution of herbicide resistance to specific modes of action is achievable.

Table 2.2 Selected herbicides for the control of *S. oleraceus.*
(Source: Pest Genie and APVMA Pubcris 2010).

Chemical Name	Mode of Action
Chlorsulfuron and Metsulfuron-methyl	Acetolactate synthase (ALS)-inhibitors
Bromoxynil and Metribuzin	Inhibitors of photosynthesis at photosystem II
Pendimethalin & Chlorthal-dimethyl	Inhibitors of tubulin formation
Diflufenican	Inhibitors of carotenoid biosynthesis
Oxyfluorfen	Inhibitors of protoporphyrinogen oxidase
2,4-D & MCPA	Disrupters of plant cell growth
Propachlor	Herbicides with multiple sites of action
Glyphosate	Inhibitors of EPSP synthase

Biological control measures

Classical biological control refers to weed management by natural enemies, such as insects or micro-organisms (Lovett and Knights 1996). Where a weed is a problem in its native environment the chances of finding a biological agent for a novel environment is reduced, although there is the possibility that an effective control agent may be present (Hutchinson *et al*., 1984). Groves (1991) cites from Julien (1982) that, at present, biological control programs have a success rate of between 25% and 40% and that the level of success is rarely predictable. Indeed, as with other single methods of control, the result may be that one weed replaces another. Peschken (1982) outlines the host specificity and biology of *Cystiphora sonchi* a candidate for the biological control of *Sonchus* species. Other than this, very few investigations into biological control of *Sonchus* species were found in the literature. This lack of information on possible biocontrol agents heightens concerns about options for controlling the weed should herbicide resistance become widespread. McCarren pers. comm. (2007) whilst searching for eriophyid mite that is common on *Sonchus* species collected seed from a number of *S. oleraceus* plants in Western Australia for use in this project.

2.3 HERBICIDE RESISTANCE

2.3.1 THE HISTORY OF HERBICIDE RESISTANCE

The potential for weeds to evolve resistance to herbicides was predicted in the 1950s (Harper 1956). Numerous weed species have now evolved resistance to herbicides (Heap and Knight 1982; Holt and LeBaron 1990; LeBaron 1991; Holt 1992; Holt *et al*., 1993; Heap 2009). Resistance has evolved in numerous weed species worldwide with 348 herbicide resistant weed biotypes of which over 100 were reported to be resistant to ALS inhibitor herbicides (Heap, 2010).

2.3.2 BIOLOGY AND ECOLOGY OF HERBICIDE RESISTANCE

2.3.2.1 Basic understanding

The evolution of herbicide resistance in weeds is influenced by the inheritance of the resistance allele, gene flow, selection pressure, plant fitness and seed bank characteristics (Tranel and Wright 2002; Gressel and Segel 1982; Powles 1993). Gressel and Segel (1982) outlined the rationale that resistance must initially be preceded by the presence of one or more alleles responsible for expressing resistant phenotypes in the population. The resistance trait can be dominant or recessive, but in most cases, a single dominant to semi-dominant gene is involved (Darmency 1994). The mechanism of herbicide resistance can be due to reduced herbicide uptake and translocation, enhanced herbicide metabolism or a modified site of action (Maneechote 1995). These are often the same mechanisms that provide selectivity to herbicides in beneficial plants (such as wheat).

2.3.2.2 Gene flow

Gene flow relates to the movement of genes among a population and this flow is capable of causing evolutionary change through the emigration (loss of genes) or immigration (gain of genes) within the population (Radosevich *et al.,* 1991). McDonald and Linde (2002) reported that organisms that have a high degree of gene flow often have greater genetic diversity than organisms with low levels. Gene flow increases the effective population size by increasing the size of the genetic pool. This will influence the maintenance of particular genotypes in a population. Gene flow processes can also directly alter the frequencies of resistant and susceptible alleles in a population (Maxwell *et al*., 1990).

Fertilisation of susceptible plants with pollen carrying alleles for herbicide resistance aids the spread of resistance (Maxwell and Mortimer 1994). This is clearly the case in plants which are mainly cross pollinated plants, such as *Medicago sativa* (alfalfa/lucerne), where the level

of gene flow is greater when plants are closer together than when they are hundreds of metres apart (Fitzpatrick *et al*., 2003). However, gene flow in self pollinated plants is mainly by 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.,* (2005a) advocate management strategies of 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 terminating the possibility of the resistance gene being transferred.

Davies and Sheley (2007) outline the fate of invasive plant seeds as either falling in the immediate vicinity of the parent plant or being dispersed by a number of vectors. In *S. oleraceus* seed is known to be widely dispersed by the wind, which consequently also disperses the resistance alleles. Understanding the mechanisms of gene flow can also be used in selecting the best weed control management methods. Likewise knowledge of the fitness between resistance and susceptible individuals can provide valuable insights as to the rate with which a resistant biotype could increase in frequency, with or without selection pressure.

2.3.2.3 Resistant biotype fitness

Mutations conferring herbicide resistance in plants are expected to produce a fitness cost in individual progeny growing in an original stress free environment (Coustau *et al.,* 2000). The fitness cost is often difficult to measure and must be compared in light of a common genetic background (Neve 2007). The relative fitness of alleles dictates the frequency with which the different alleles will be present within the population. For the particular case of herbicide resistance, Holt and LeBaron (1990) concentrated on parameters such as growth rate and plant biomass as indicators of fitness. In the presence of herbicide, herbicide resistant plants have a fitness advantage over susceptible plants. However, in the absence of herbicide the opposite

may occur. Herbicide resistance alleles are kept at low frequencies in populations in the absence of herbicide by the fitness penalty against the resistance allele (Jasieniuk *et al.,* 1996). Neve (2007) emphasises the need to have a good understanding of population differences that may have nothing to do with the presence or absence of the resistance allele.

Susceptible plants in the population are often classified as standard "fit" biotypes whereas resistant plants after herbicide has been applied may be classified in classes of fitness from 1, (the least fit) to 5, (the most fit) or equal to the non sprayed control. Widderick (2002) noted differences in *S. oleraceus* fitness traits and placed *S. oleraceus* plants, into 3 bands of fitness (after applying herbicide and measuring survival), as 1, 2 and 3, with 3 being the most fit. Other metric scales relating to the fitness level in resistance have included % R (% Resistant plants) and % Intermediate (% Intermediate resistant plants) (Hashem and Bowran 2001; Boutsalis and Powles 1995a). However, these classes do not allow for non-linear variations in the level of resistance or comparisons of total resistance equal to the fitness of the non-sprayed control. Two fundamental components of fitness are survival and reproduction (Silvertown 1987). Often the fitness of a weed is determined by its biomass production (Holt 1992), although this provides no indication of the ability to reproduce. In addition other genes in the plant can influence the fitness of the plant, such that an individual carrying a resistance allele may be more fit, as fit or less fit than any one individual carrying a susceptible allele.

2.3.2.4 Inheritance of herbicide resistance

In the majority of cases studied the inheritance of resistance is by a single partially dominant or dominant gene (Anderson and Gronwald 1987; Boutsalis and Powles 1995a; Betts *et al,.* 1992; Barr *et al,.* 1992; Mallory-Smith *et al.,* 1990b; Itoh and Miyahara 1984; Shaaltiel *et al.,* 1988; Islam and Powles 1988 and Purba *et al.,* 1993). An exception is the resistance to dinitroanaline herbicides in *Setaria viridis* where resistance is due to a single recessive gene

(Jasieniuk *et al.,* 1994). Inheritance of resistance in all classes of herbicides except the triazines is by nuclear inheritance. In the majority of plants with resistance to triazine herbicides, resistance is inherited cytoplasmically through maternal inheritance (Jasieniuk *et al.,* 1996).

Mutations in ALS conferring herbicide resistance are at least partially dominant, and because the gene is nuclear inherited, it is transmitted by both seed and pollen (Saari *et al.*, 1994; Tranel and Wright 2002). Boutsalis and Powles (1995a) crossed a chlorsulfuron resistant biotype of *S. oleraceus* with a susceptible biotype and followed the inheritance through F_1, F_2, and F_3 generations. F_1 hybrids from this cross were uniformly resistant, but at an intermediate level compared to the resistant and susceptible parents. Following the application of chlorsulfuron herbicide to the F_2 generation, three distinct phenotypes were identified, resistant, intermediate and susceptible. A segregation ratio of 1:2:1 was observed indicating a single, nuclear, incompletely dominant gene conferring resistance. F_3 families also segregated in a 1:2:1 ratio. They concluded that resistance to herbicides inhibiting ALS in this biotype of *S. oleraceus* is due to the effect of a single gene coding for a resistant form of the target enzyme, ALS. Ashigh and Tardif (2007) conducted similar work investigating the genetics of resistance to ALS-inhibiting herbicide imazethapyr in the self-pollinated plant *Solanum ptychanthum* (eastern black nightshade). Their backcross progenies of the F_2 families also segregated in the ratio of 1:2:1 (Resistant, Intermediate and Susceptible) agreeing with earlier work of Boutsalis and Powles (1995a).

2.3.2.5 Cross resistance

Cross resistance is said to exist in weed populations when the plants exhibit resistance to a herbicide that has never been applied to the population (Heap and Knight 1986; Holt *et al.,* 1993). In other words, the weed population or biotype has evolved resistance through a

mechanism that allows resistance to another herbicide. Cross resistance has reported to be common within the sulfonylurea herbicide family. For example Holt *et al*., (1993) reported that chlorsulfuron resistance will also result in a population or biotype that is resistant to all the other sulfonylurea herbicides.

2.3.2.6 Multiple resistance

Multiple resistance is said to exist in a weed population when it exhibits resistance to dissimilar herbicide modes of action through more than one resistance mechanism (Powles and Mathews 1991). In other words, multiple resistance refers to a weed biotype that has evolved mechanisms of resistance to more than one herbicide often brought about by separate selection processes. An example of this would be if an *S. oleraceus* biotype resistant to ALS-inhibiting herbicide was found to also be resistant to inhibitors of photosynthesis at photosystem II, that is, resistant to chlorsulfuron and bromoxynil.

2.3.2.7 The soil seed bank

Liebman *et al*., (2007) reports that if cultural practices keep seed banks at negligible levels then a species may eventually be eliminated from a field, although this may take some time for species with highly persistent seed banks. The longevity of the seed within the seed bank and the size of the seed bank play important roles in population dynamics of herbicide resistant weeds (Holt and Thill 1994), because a long lived seed bank acts as a buffer against the rapid evolution of herbicide resistance. Repeated application of the same herbicide to a weed population will continuously enrich the seed bank with resistant seed (Gressel and Segel 1982). *S. oleraceus* emerges all year round, has low levels of seed dormancy and emergence is favoured by minimal tillage systems (Widderick 2002), so the genetic composition of the seed bank changes rapidly. Hence, it could be anticipated that there will be rapid selection for herbicide resistant biotypes with repeated applications of the same herbicide.

2.3.2.8 Herbicide resistance management

Zollinger and Parker (1999) report that *Sonchus* spp. are pioneer species, invading natural habitats and disturbed sites, are long travelled by wind dispersion and their rapid germination allows establishment in diverse habitats. This produces a challenge for the management of a wind dispersed weed. Herbicide mode of action rotations and the use of herbicide mixtures are methods of reducing resistant population build up (Gressel 1991 and Slife 1986). Integrated Weed Management Strategies (IWMS) involve controlling weeds by using combinations of biological, physical or chemical methods (Powles 1993 and Mathews 1994). These include methods such as rotating herbicide mode of action, rotating crop varieties, utilising cultural weed control techniques, reducing weed seed contamination, crop and pasture topping and monitoring paddocks (CRC Australian Weed Management 2006). Neve (2007) calls for a greater integration of 'evolutionary-thinking' into herbicide resistance research with a need for weed scientists to become less focused on simply describing resistance and more driven towards a deeper understanding of the evolutionary forces that underpin resistance evolution. In addition Neve (2007) suggests that the major research effort should be towards the development of economically viable strategies to prevent and manage resistance. Research into the precise details of biochemical, genetic and molecular means by which plants evolve herbicide resistance will contribute to wiser use of herbicides, new innovations and better strategies for weed management (Powles and Yu 2010). It is hoped that the outcome of this research will provide strategies to prevent and manage resistance in a wind dispersed weed.

2.3.3 ACETOLACTATE SYNTHASE (ALS)-INHIBITING HERBICIDES

2.3.3.1 Acetolactate synthase–inhibiting herbicides

In plants, acetolactate synthase (ALS) is the first enzyme in the biosynthetic pathway for the branched chain amino acids valine, leucine and isoleucine (DeFelice *et al.,* 1974). The

enzyme acetolactate synthase is the target for the ALS-inhibiting herbicides. These herbicides are very potent, are applied at low rates, are selective in their action in plant species and have low mammalian toxicity (Beyer *et al.,* 1988 and Shaner *et al.,* 1982). These properties have ensured that ALS-inhibiting herbicides are widely used worldwide but the intensive use of these herbicides has resulted in the selection of resistance in weed populations.

2.3.3.2 ALS-inhibiting herbicide classification

Mallory-Smith and Retzinger (2003) outlined a revised classification of herbicides by site of action for weed resistance management strategies following their original classification of 1997 (Retzinger and Mallory-Smith 1997). They classified the herbicides according to the primary site of action in the plant. Within the ALS-inhibiting herbicides there are 5 chemical families (Green *et al.,* 2009)

1. Sulfonylureas
2. Imidazolinones
3. Sulfonamides (Triazolopyrimidines sulfonamides)
4. Pyrimidinylthiobenzoates
5. Sulfonylamino-carbonyl-triazolinones

2.3.3.3 The sulfonylurea chemical family

The majority of the herbicide resistance work with ALS-inhibiting herbicides has been focused on herbicides within the sulfonylurea chemical family. Table 2.3 outlines the ALS-inhibiting herbicides currently used in Australia, the weeds they control and the crops in which they are used.

Table 2.3 Group B herbicides in the sulfonylurea family used in Australia.

Herbicide	Trade names	Weeds controlled	In Crops
Sulfonylureas			
Metsulfuron-methyl	Ally® Associate®	Broadleaf weeds	Wheat, barley and triticale
Mesosulfuron-methyl	Atlantis®	Brome grass, wild oats, annual ryegrass and annual phalaris	Wheat
Ethametsulfuron-methyl	Bounty® with diflufenican	Broadleaf weeds	Lupins
Trifloxysulfuron-sodium	Envoke®	Broadleaf weeds and nutgrass	Cotton
Tribenuron-methyl	Express®	Broadleaf weeds	Fallow and pre crop
Chlorsulfuron	Glean® Lusta® Siege®	Broadleaf weeds and some annual grasses	Wheat, barley, oats and triticale
Thifensulfuron-methyl	Harmony®M with metsulfuron-methyl	Broadleaf weeds	Wheat, barley and triticale
Iodosulfuron-methyl-sodium	Hussar®	Broadleaf weeds, wild oats and annual phalaris	Wheat
Triasulfuron	Logran® Nugran®	Broadleaf weeds, annual ryegrass, paradoxa grass	Wheat, barley, oats and triticale
Bensulfuron-methyl	Londax®	Arrowhead, dirty dora, starfruit	Rice
Sulfosulfuron	Monza®	Brome grass and some broadleaf weeds	Wheat
Sulfometuron-methyl	Oust®	Broadleaf weeds and grasses	Non crop situation
Halosulfuron-methyl	Sempra®	Nutgrass	Cane, maize and sorghum
Rimsulfuron	Titus®	Some broadleaf weeds	Tomato
Azimsulfuron	Gulliver®	Aquatic weeds	Rice

Source: (Preston pers. comm. 2009)

2.3.4 RESISTANCE TO SULFONYLUREA HERBICIDES

2.3.4.1 Background

In 1987, Mallory-Smith *et al.,* (1990a) described resistance to ALS-inhibiting herbicides in prickly lettuce (*Lactuca serriola* L.), five years after the commercial introduction of chlorsulfuron. Since 1987 the number of weed species recorded as showing resistance to ALS-inhibiting herbicides has increased to 101 worldwide (Heap 2010). ALS-inhibiting herbicides have been widely adopted due to their low rates, broad spectrum of weed control,

low mammalian toxicity and flexible application timing in a many crops (Mazur and Falco 1989; Duran-Prado *et al*., 2004; Saari *et al*., 1994).

2.3.4.2 ALS genes and mutations

Mutations in ALS conferring herbicide resistance are at least partially dominant, and because the gene is nuclear inherited, these mutations are transmitted by seed and pollen (Tranel and Wright 2002). Grula *et al.,* (1995) reported that all of the plant ALS genes sequenced to date lack introns. Genetic studies have shown the ALS gene to be encoded by a single copy in some species (Haughn and Sommerville 1986) and as two active ALS genes in tobacco (*Nicotiana tabacum*) (Lee *et al.,* 1988). The allotetraploid, *Brassica napus* has several ALS genes (Rutledge *et al.,* 1991). Work of Guttieri *et al.,* (1995) and Bernasconi *et al.,* (1995) found that amino acid substitutions exist at five sites (Alanine$_{122}$, Proline$_{197}$, Alanine$_{205}$, Tryptophan$_{574}$ and Serine$_{653}$) or domains (identified A to E) in the gene and that these domains are mutation sites. A sixth (Aspartate$_{376}$,) and seventh (Glycine$_{654}$,) site have since been identified (Whaley *et al*., 2007) and (Sales *et al*., 2008). In 2009 there were 22 resistance substitutions at seven sites across ALS (Powles and Yu 2010).

2.4 GENETICS AND EVOLUTION

2.4.1 INTRODUCTION

The method of pollination in plants makes considerable difference to the genetic diversity of the progeny. Many plants are cross pollinated, which leads to increased genetic variation. However, some plants are self pollinated, which leads to more uniform progeny (Lawrence 1974). *S. oleraceus* is self pollinated and therefore produces more uniform progeny.

Stankiewicz *et al.,* (2001) stated that herbicide resistance in weeds is an example of micro-evolution in plant species caused by environmental changes brought about by human activity

and is a result of selection for traits that allow a weed species to survive. Herbicide resistance in plants most often occurs as a single dominant allele, therefore phenotypic resistant plants will have the genotypes of either RR or Rr and susceptible plants will have the genotype of rr. The pattern of transmission of alleles to progeny will be affected by the method of pollination. Therefore, understanding the pollination biology of the target species can help in choosing an approach for a study of gene flow (Baker *et al.,* 2005).

2.4.2 POPULATION GENETICS

Populations vary in their number or density per unit area depending on the level of availability of nutrients, water and environmental constraints such as temperature and light. Phenotypic variations in the population can exist or be caused by novel genetic mutations. Populations and the individuals within populations vary in both their genotype and phenotype and the frequency of alleles will vary depending on the selecting pressure applied (Russel 1980). Geographic isolation can result in significant genotypic divergence, although this will be influenced by the founding genotypes. For example, populations of one-leaf tulip (*Moraea flaccida*) collected in South Australia less than 136 km apart were found to be genetically similar, whereas populations in Western Australian and South Australian were genetically different (Blackman 2005).

2.4.2.1 Hardy-Weinberg equilibrium

Within a defined geographical area and in the absence of genetic drift or significant immigration, but where mating is at random, a gene pool contains R alleles at a frequency of p and r alleles at a frequency of q. The gene pool will remain in equilibrium $p^2 + 2pq + q^2 = 1$ as long as these conditions continue. This means that gene frequencies and genotype ratios in a randomly-breeding population remain constant from generation to generation, following what is known as the Hardy-Weinberg equilibrium. Selection processes affect the survival of

individuals in the population and evolutionary changes, such as the development of herbicide resistance occur altering this equilibrium (Russel 1980).

2.4.2.2 Selection Pressure

The initial frequency of alleles can differ in populations. When no selection pressure is applied, the frequency of alleles for herbicide resistance is usually low (Neve and Powles 2005b). Herbicides provide a high level of selection pressure on a weed population (Jasieniuk *et al*., 1996) and the stronger the selection pressure, the faster the emergence of resistance (Gressel and Segel 1982). A ten times increase in the frequency of resistance alleles in the population is produced by a 90% kill with the use of a herbicide (Howat 1987). However, Preston and Roush (1998) point out that herbicides applied at a low rate may slow the rate of resistance evolution in weed populations because fewer susceptible weeds are killed.

2.4.2.3 Genetic drift and genetic distance

Genetic drift is a random change in allele frequency (Russel 1980). This drift can be small in large populations, but large in small populations because of the number of alleles present in the gene pool. That is, the level of original heterozygosity will remain high in large populations. Small populations respond differently as a result of random matings and the number of offspring from each successful mating over succeeding generations (Strickberger 1968). In the case of small populations, rare alleles are often lost purely by chance reducing the heterozygosity over time.

2.4.2.4 Inbreeding and fixation index (F_{ST})

In individual plants, inbreeding redistributes alleles from the heterozygous to the homozygous state. Although the genotype frequencies may be changed under Hardy-Weinberg equilibrium, the allele frequencies are unaltered (Futuyma 1998). Fixation index (F_{ST}) is

often used as a measure of the observed variation in allele frequencies among populations. Although it must be noted that the estimate obtained in one population cannot be compared with that of another, unless the breeding system is similar for the two populations (Nei 1973). F_{ST} can be interpreted as the averaged standardized variances of all allelic variants. The differences in the allele frequencies among populations allow us to infer the level of gene flow by assuming the more similar the allele frequencies the higher the rate of gene flow. However, two assumptions must also hold true: the alleles measured must be selectively neutral and the allele frequencies must have reached an equilibrium between genetic drift and gene flow. Although in general the magnitude of gene flow estimated from genetic data corresponds fairly well with what may be expected from the natural history of the species (Futuyma 1998).

2.4.2.5 Founder effect

The founder effect commences from the initiation of a new population with new founding genes (Futuyma 1998). Essentially, without immigration and spontaneous mutations, those alleles present in the founding individuals will determine the alleles for the future population. Blackman (2005) found evidence of a founder effect in a closed population of one-leaved tulip in South Australia. The presence of different founder germplasm may provide support for multiple introductions of *S. oleraceus.*

2.4.2.6 Bottlenecks

Bottlenecks in populations arise when the population is temporarily restricted in size, either by a new colonisation event or a natural disaster (Futuyma 1998). They occur when the size of a new population becomes so small that a genetic bottleneck is formed (Russel 1980). Under these conditions genetic drift may counteract natural selection and fix an allele purely by chance.

2.4.2.7 Seed bank characteristics

The state of the seed bank can influence the rate of resistance evolution and the longer the seed life of susceptible biotypes the greater the buffering effects in the seed bank, resulting in a decreased rate of resistance evolution (Hidayat 2004). Since *S. oleraceus* has low seed dormancy (Widderick 2002) changes in the gene pool can move at a rapid rate.

2.4.3 MOLECULAR GENETICS AND RESITANCE EVOLUTION

2.4.3.1 Introduction

Molecular genetics is the study of the regulation of genetic information at the level of DNA, RNA and protein molecules. The *in vitro* amplification of DNA by the Polymerase Chain Reaction (PCR) has proven to be a revolutionary technique in molecular biology (Phillips and Vasil 2001; Clark 2005). Molecular biotechnology emerged as a new research field in the late 1970s as the result of this fusion of recombinant DNA technology and traditional industrial microbiology (Glick and Pasternak 2003). In 1944, Avery, MacLeod and Mc Carthy demonstrated that DNA is the genetic material and in 1953, Watson and Crick determined the structure of DNA. From 1961 to 1966 the entire genetic code was deciphered. Through the 1970s there was the establishment of recombinant DNA technology. In the 1980s and 1990s further DNA, PCR technology and methods were established leading to genetically modified crops, the nuclear cloning of a sheep and more importantly the revolution in population studies as the methods provided the opportunity to rapidly assess genetic changes without extensive breeding programmes.

Molecular techniques are now widely used as a tool in herbicide resistance evolution, for example Prado *et al.,* (2004) outlined several mechanisms leading to resistance to ALS-inhibiting herbicides, with the most important being the presence of nuclear–inherited dominant mutations in the DNA sequence coding for the ALS enzyme. In wild plants, these

mutations are located in different conserved domains where the herbicide binds to the enzyme (Boutsalis *et al.,* 1999).

2.4.3.2 Molecular components, techniques and methods

Molecular markers and polymorphism

A polymorphism is a genetic variant that results in two or more clearly different phenotypes existing in the same population of a species. Phillips and Vasil (2001) outline some new methods for molecular maps in plants and point out that there are widespread polymorphisms in natural populations. Each plant has variations in DNA sequences in their genome and this variation can be detected using genetic markers (Sunnucks 2000).

PCR markers

Polymerase Chain Reaction (PCR) is a laboratory test tube technique to amplify specific DNA sequences by the extension of primers on DNA strands (Taylor 1991). For example, Wagner *et al.,* (2001) discuss the adaptation of the PCR amplification of specific alleles (PASA) for detection of point mutations in *Amaranthus retroflexus* and *Amaranthus rudis* leading to ALS inhibitor resistance. The major advantages of the method include the fast and clear test of the young plants for mutations. This test is a valuable tool for the identification of ALS-inhibiting resistance mutations.

RFLP

Restriction fragment length polymorphism (RFLP) uses a restriction endonuclease to digest genomic DNA to form different fragments of DNA as a result of variations in DNA sequence (Whitkus *et al*., 1994). This marker technique detects polymorphisms in the genome of individuals (Jones *et al.,* 1997).

AFLP

Amplified Fragment Length Polymorphism (AFLP) is a fingerprinting technique increasingly used to study genetic variation in plants (Vos *et al*., 1995). As a molecular tool it is rapid and a relatively simple method that can be applied to give much information about the variation present within a population (Baker 2002).

RAPD

Randomly amplified polymorphic DNAs (RAPDs) are DNA fragments generated by the polymerase chain reaction using primers (Williams *et al.,* 1990). RAPD analysis has been criticised due to user error, which makes results between different laboratories difficult to reproduce. However, they are simple and require no knowledge of the genome sequence (Baker *et al.,* 2005).

SSRs or microsatellites

Microsatellites or simple sequence loci are mainly distributed in genomic DNA in all regions of the chromosomes (Lu 2005). SSRs and microsatellites are probably the marker of choice for marker-based genetic analysis and marker-assisted plant breeding (Akkaya *et al*., 1992). Akkaya *et al*., (1992) reports that acceptable numbers of polymorphisms are observed with SSRs in self pollinated plants, whereas the number of polymorphisms detected by RFLPs is prohibitively low.

ISSR

Inter simple sequence repeats (ISSRs) have been used in numerous plant research areas including genetic diversity, phylogenic studies, gene tagging, genome mapping and evolutionary biology of different plant species (Godwin *et al*., 1997). ISSRs are relatively simple repeatable and can handle a large number of samples (Blackman 2005).

Dominance issues

McDonald and McDermott (1993) outline that molecular markers are either dominant or co-dominant. Dominant systems such as randomly amplified polymorphic DNAs (RAPDs) and amplified fragment length polymorphisms (AFLPs) are unable to differentiate between heterozygous (Rr) and homozygous (RR rr) individuals since the markers are identified as present or not present.

2.4.4 SUMMARY - ISSUES IN ECOLOGICAL EXPERIMENTS

Issues such as genetic variation, environmental variation and changes in ecological characteristics will be considered in selecting the molecular method and statistical analysis method for interpreting genetic diversity and gene flow in this study. Differences in allele frequency detected between populations elucidate the reasons for these observed differences, based on all known information in each particular case (Baker 2002).

When collecting samples there is always the element of thought as to whether the number of samples collected will yield meaningful data relating to differences. If the alleles of interest are rare then large numbers of individuals are required to detect its presence (Roush and Miller 1986). However, these authors further found that to observe a band (allele) that occurs at a frequency of 0.1% with a confidence level of 60%, only 10 individuals are be required. Certainly sampling a large percentage of the population is logistically impossible and, if it is accepted that rare alleles may be missed, representative samples may give indications of the population's genetic makeup. This study is more concerned with genetic variability on a landscape scale than on the detection of rare alleles, therefore, collections of a small number of individuals have been made from a wide geographical area and will be used to establish the presence of herbicide resistance and the genetic similarity between the individuals.

In conclusion this literature review has outlined a broad spectrum of what is known of the biology and ecology of *S. oleraceus*, aspects of herbicide resistance, population genetics and molecular genetics. What is not known is the distribution of resistance alleles in *S. oleraceus* in Australia, how the genes move, the genetic diversity of *S. oleraceus* and the sensitivity of *S. oleraceus* to the ALS-inhibiting herbicide, chlorsulfuron. These unknowns are the basis of this investigation.

CHAPTER 3

3 GENERAL MATERIALS AND METHODS

3.1 *Sonchus oleraceus* SEED COLLECTION IN AUSTRALIA

Populations of *S. oleraceus* were collected from throughout Australia between 1997 and 2008 (Figure 4). The accessions collected are listed from So1 to So574 in Appendix 1. The list includes the collection date, location and whether tested for resistance or susceptibility to chlorsulfuron. So1 to So40 were collected in 1997 and 1998 (Widderick 2005). New collections for this study commenced with So101 from a railway line in Wasleys, South Australia. Subsequent collections of *S. oleraceus* were made in southern Queensland and northern New South Wales, in areas where the species was known to have evolved resistance to ALS-inhibiting herbicides, and in other areas in Australia (Figure 3.1) to find how widespread herbicide resistance was. Seed collection details are outlined in Section 3.2. The first recorded case of resistance to ALS-inhibiting herbicides in *S. oleraceus* was found in 1991 at Goondiwindi (Figure 3.1) (Boutsalis and Powles 1995a). Subsequent surveys from 1997 to 2008 collected more accessions from locations in Australia (Figures 3.2 to 3.7). A total of 274 accessions from across Australia were treated with the ALS-inhibiting herbicide, chlorsulfuron to determine the level of resistance to the herbicide on a state by state basis.

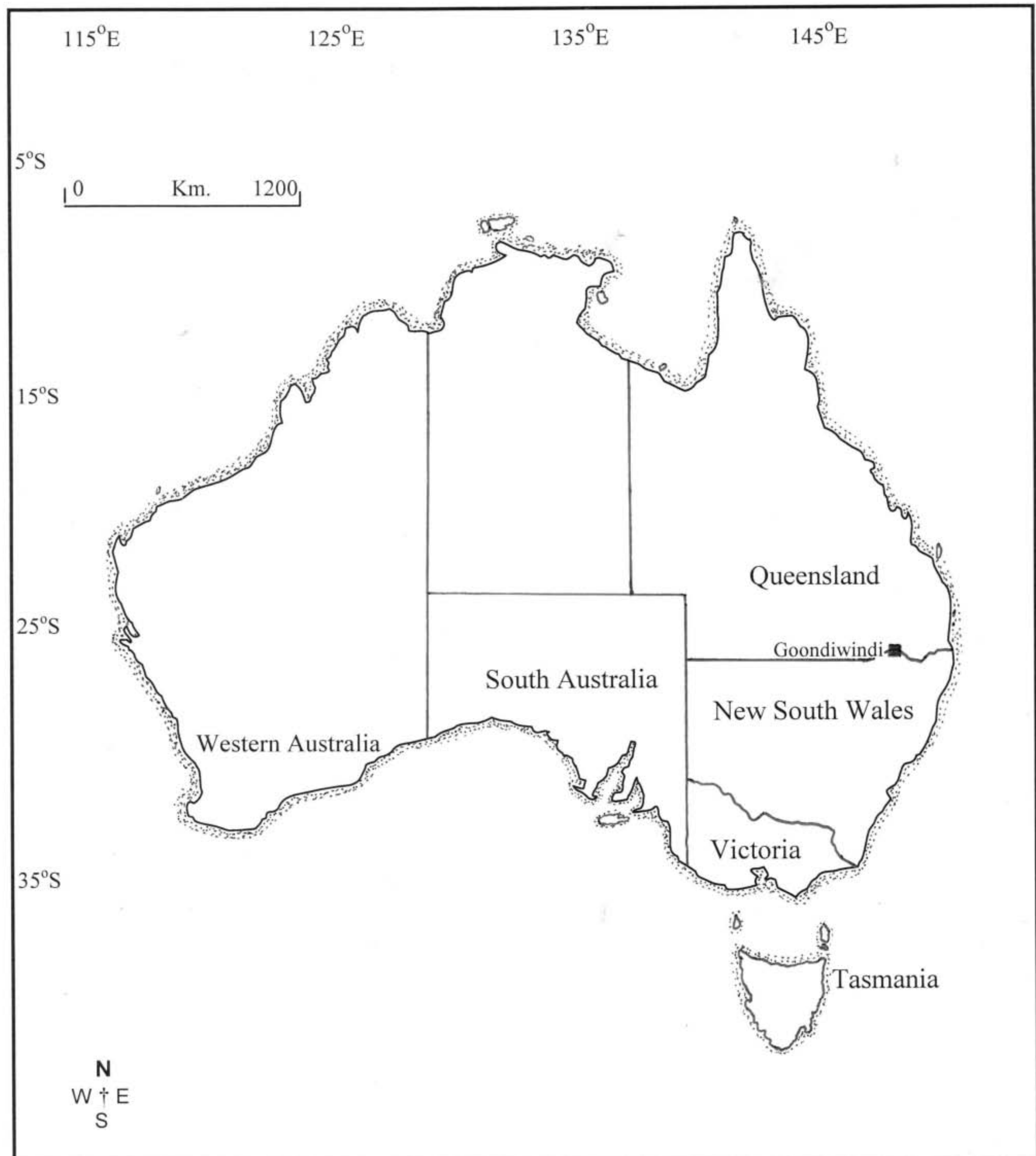

Figure 3.1 General location of seed collections of *S. oleraceus* made in Australia between 1997 and 2008. The collection site of the first *S. oleraceus* (sowthistle) resistant to ALS-inhibiting herbicide found at Goondiwindi, Queensland in 1991 is also displayed.

3.1.1 QUEENSLAND

Figure 3.2 shows the location of Goondiwindi, where the first recorded case of resistance to ALS-inhibiting herbicides in *S. oleraceus* was found in 1991 (Boutsalis and Powles 1995a). The location of collection sites in Queensland, north of Goondiwindi are also shown. Ninety eight accessions from Queensland were treated with the ALS-inhibiting herbicide, chlorsulfuron to determine the level of resistance to the herbicide.

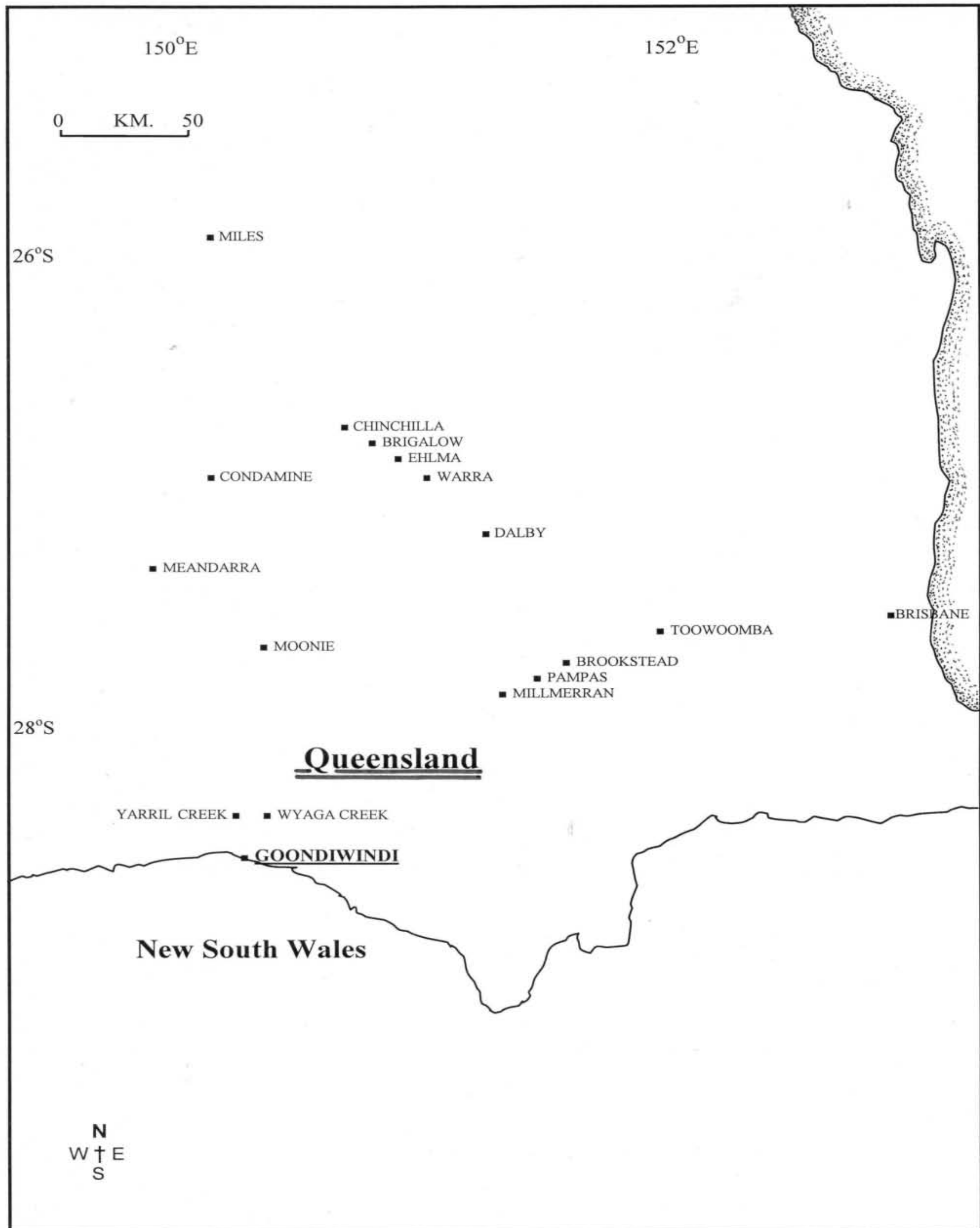

Figure 3.2. Distribution of *S. oleraceus* (sowthistle) collection sites in Queensland Australia.

3.1.2 NEW SOUTH WALES

The location of collection sites south of Goondiwindi, in New South Wales (Figure 3.3). Thirty two accessions from New South Wales were treated with the ALS-inhibiting herbicide, chlorsulfuron to determine the level of resistance to the herbicide.

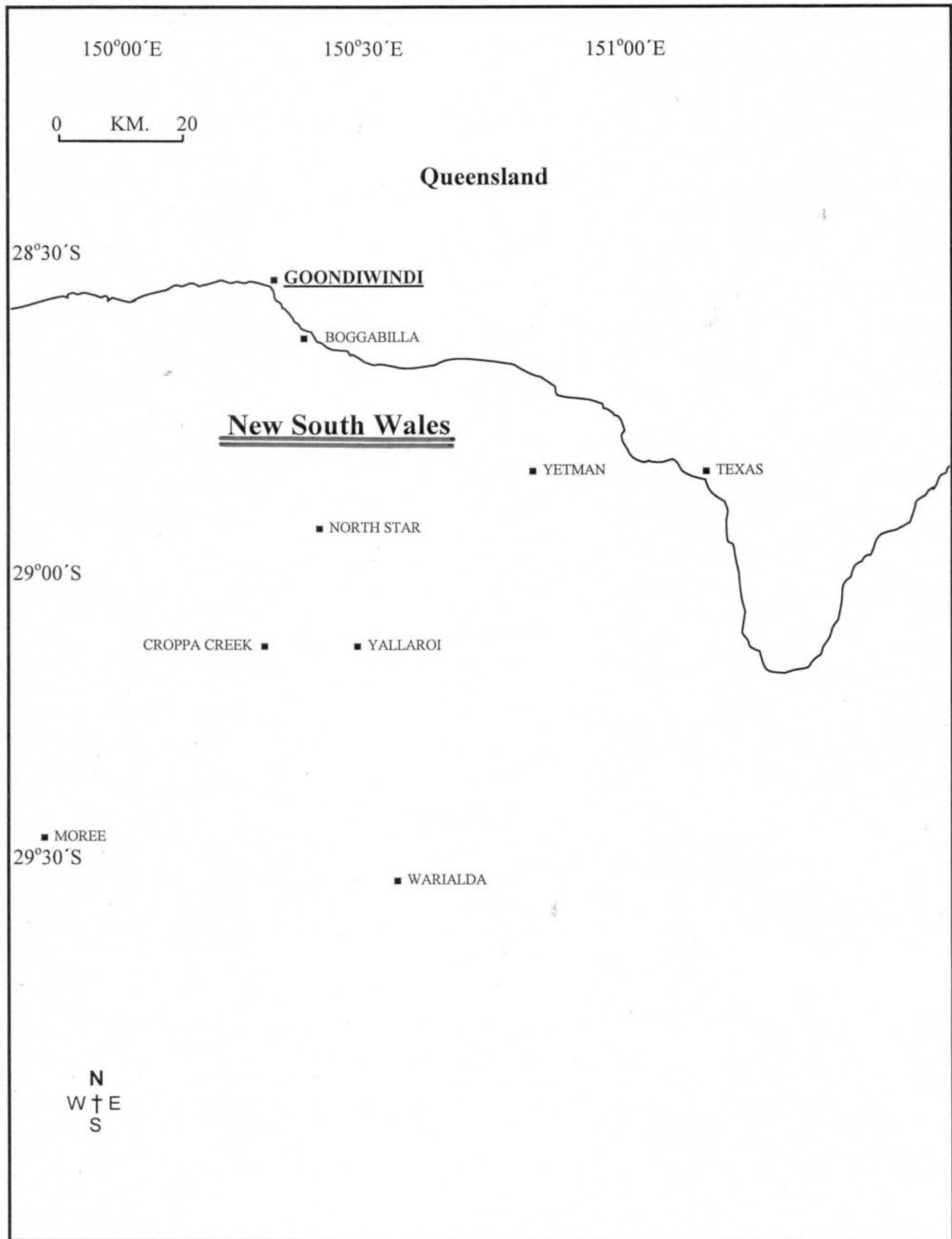

Figure 3.3 Distribution of *S. oleraceus* (sowthistle) collection sites in New South Wales Australia.

3.1.3 SOUTH AUSTRALIA

The first sample collected in South Australia for this project was collected between railway lines at Wasleys (Figure 3.4) accession number So101. It was later screened and found to be resistant to the ALS-inhibiting sulfonylurea herbicide chlorsulfuron. The resistance is likely

to have evolved from the railway utilities continuous use of the sulfonylurea herbicide sulfometuron methyl. Other locations of collection sites in South Australia are shown. One hundred and nineteen accessions were treated with the ALS-inhibiting herbicide, chlorsulfuron to determine the level of resistance to the herbicide.

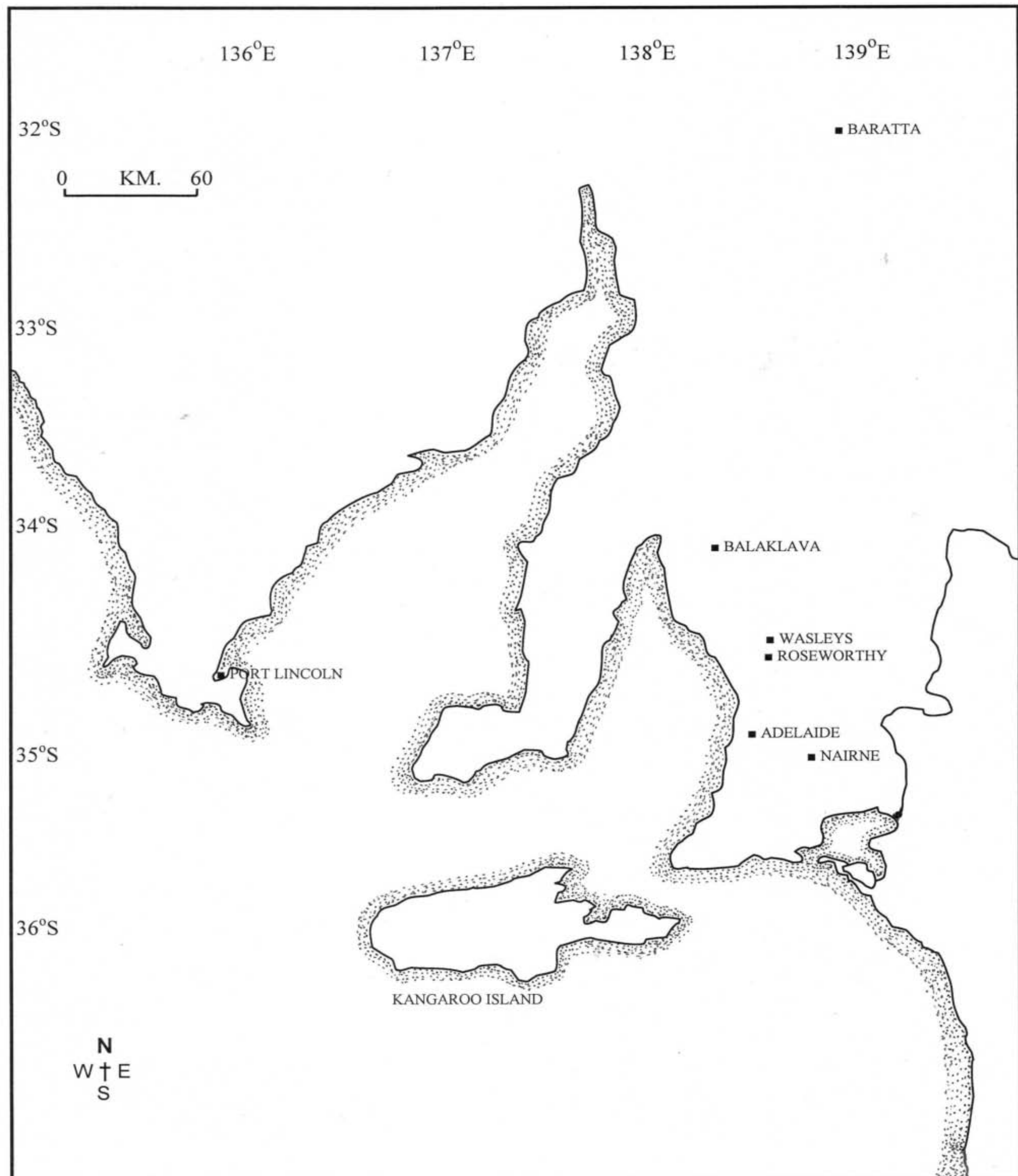

Figure 3.4 Distribution of *S. oleraceus* (sowthistle) collection sites in South Australia, Australia.

3.1.4 WESTERN AUSTRALIA

The location of seed collected by Dr. Kathryn McCarren of CSIRO Entomology, Wembley, W.A. whilst surveying for eriophyid mite on *Sonchus* (Figure 3.5). Twelve accessions from

this collection were treated with the ALS-inhibiting herbicide, chlorsulfuron to determine the level of resistance to the herbicide.

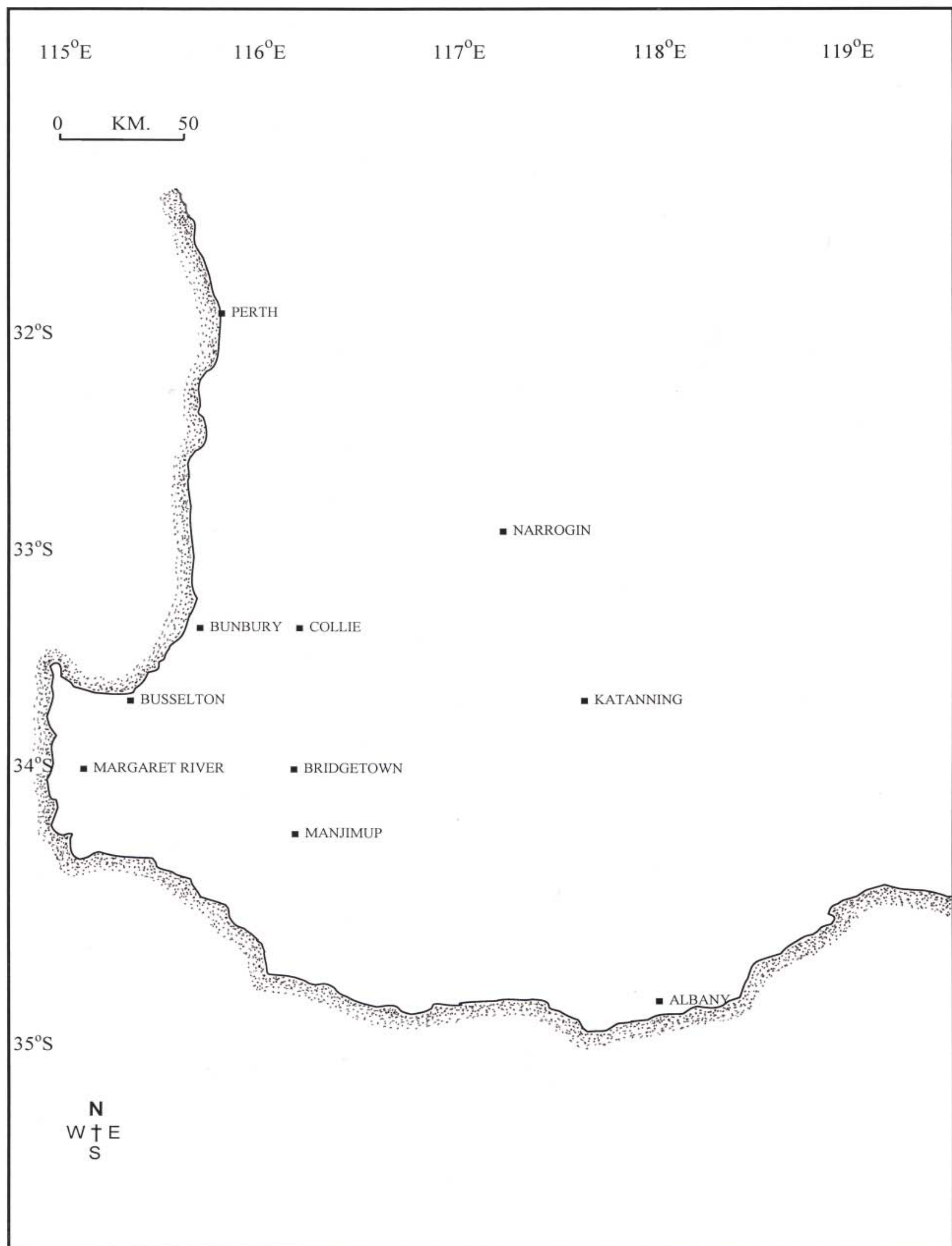

Figure 3.5 Distribution of *S. oleraceus* (sowthistle) collection sites in Western Australia, Australia.

3.1.5 TASMANIA

Figure 3.6 shows the collection site at Roaches Beach, near Hobart where the only *S. oleraceus* plant was collected in Tasmania.

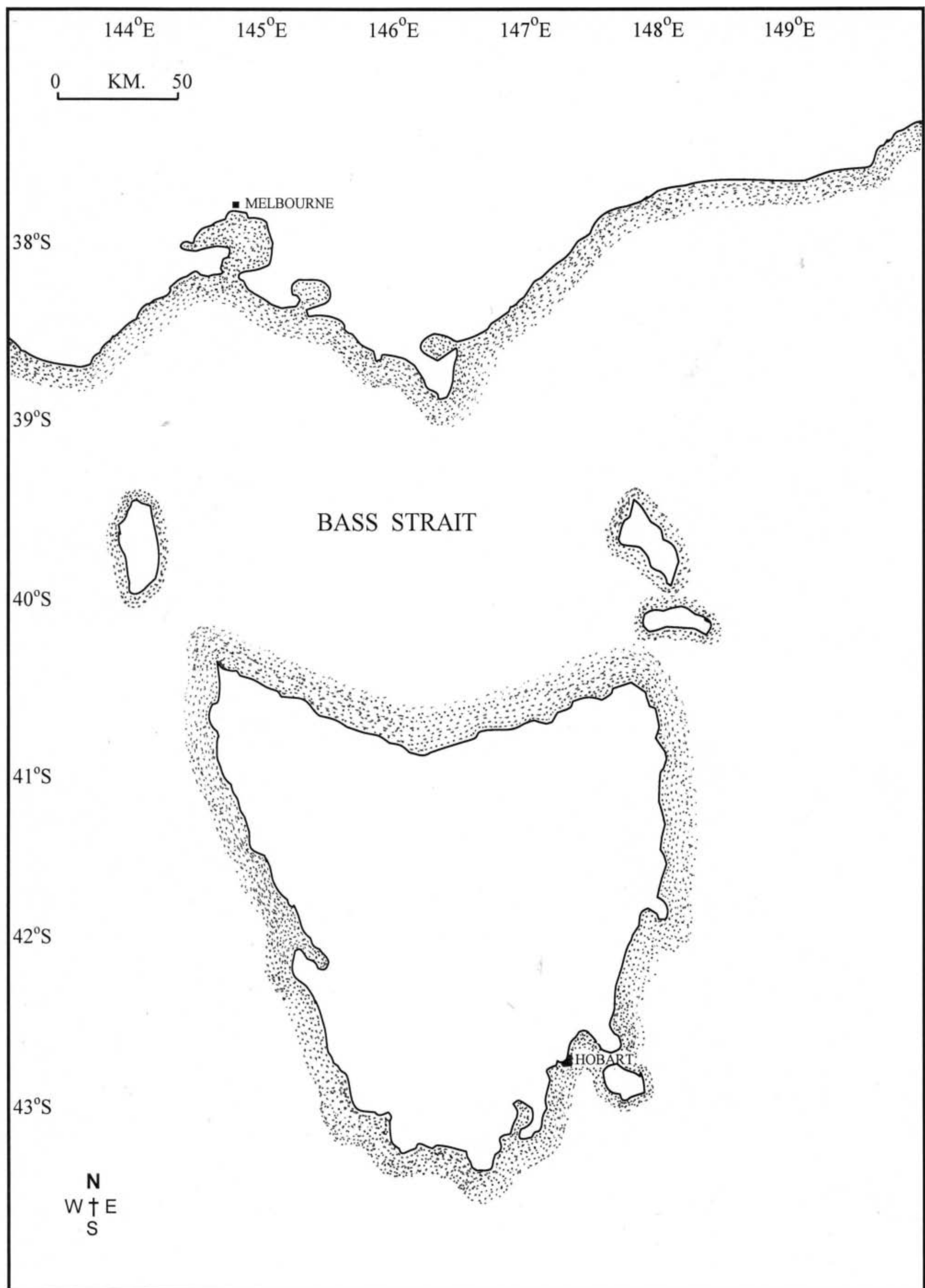

Figure 3.6 *S. oleraceus* (sowthistle) collection site at Roaches Beach, 15 km east of Hobart in Tasmania, Australia.

3.1.6 VICTORIA

Location of seed collected by Dr. Peter Boutsalis of the University of Adelaide whilst surveying herbicide resistant weeds in Victoria (Figure 3.7). Twelve accessions from Victoria were treated with the ALS-inhibiting herbicide, chlorsulfuron to determine the level of resistance to the herbicide.

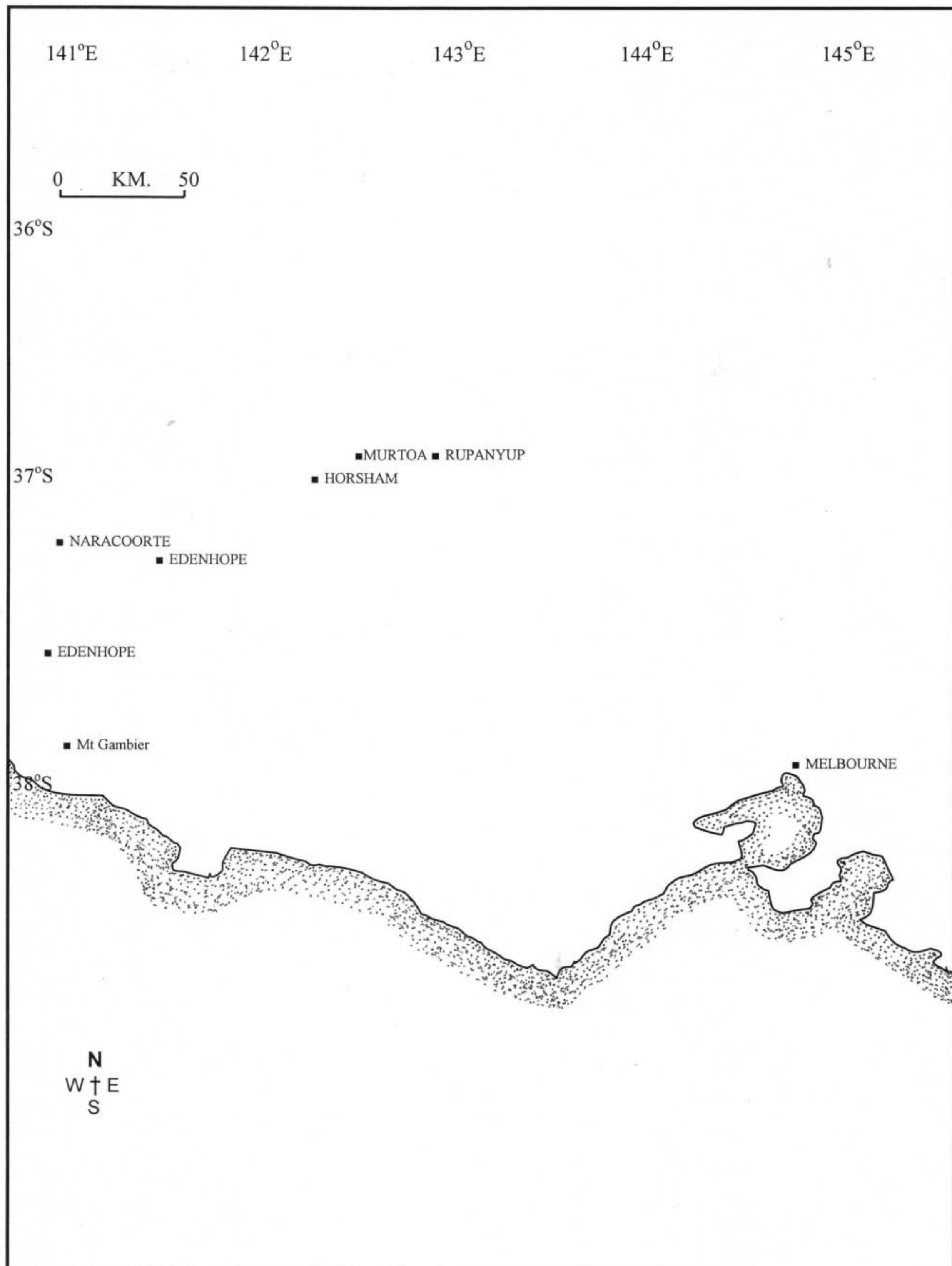

Figure 3.7 Distribution of *S. oleraceus* (sowthistle) collection sites in Victoria, Australia.

3.2 SEED COLLECTION AND STORAGE

3.2.1 SEED COLLECTION AND DRYING

Accessions were created by collecting seed from single plants. The number of seed heads collected from each plant varied from one to approximately 20. Seed was collected directly into a seed envelope. Envelopes were labelled with the *S. oleraceus* number, GPS coordinates and the date and time. Seed was allowed to dry naturally or if necessary dried in a fan forced dehydrator at 35°C for 24 hrs.

3.2.2 SEED STORAGE

Labelled envelopes of dry seed were placed in water proof plastic containers and stored in a cold room at 3°C until required.

3.3 GERMINATION, CULTIVATION AND PLANT TREATMENTS

3.3.1 GERMINATION

Seeds were germinated on 0.6% (w/v) agar in a controlled environment cabinet with a 12 hour light period at 20°C at 30 μmol $m^{-2}s^{-1}$ during the day and 12 hours dark period at night for seven days. Seedlings of even size and vigour were transplanted at the cotyledon stage (Figure 3.8) to cell trays (30 mm x 30 mm cells with 9 rows and 22 columns, each cell being tapered to a depth of 50 mm) or 0.55 L MK12 MasracTaglok punnet pots (90 x 80 mm) containing coco peat potting mix. Coco peat potting mix was produced by mixing 540 L of coco peat, 220 L of water and 60 L of sand prior to steaming for 1 hour. The following additives were then mixed to the pasteurised mix; 180 g Dolomite lime; 600 g Agricultural lime; 240 g Hydrated lime; 180 g Gypsum; 180 g superphosphate; 450 g iron sulphate; 30 g iron chelate; 180 g Micromax trace elements; 450 g Calcium nitrate and 1800 g of Osmocote mini 3-4m (16-3-9+te).

3.3.2 PLANT GROWTH CONDITIONS

Cell trays or punnet pots were transferred to the temperature controlled (21°C) glasshouse and watered with a mist spray as required.

3.3.3 LEAF SAMPLING FOR DNA EXTRACTION

Leaf material for DNA extraction, 1cm^2, was cut with sterile scissors from plants and transferred to a sterile labelled 1.5 mL eppendorf tube on ice. DNA was extracted immediately as section 3.4.1. After leaf sampling, plants were treated with the herbicide, chlorsulfuron to determine whether they were resistant or susceptible to the herbicide (as section 3.3.4).

3.3.4 HERBICIDE TREATMENT AND MEASUREMENTS

At the two-leaf stage (Figure 3.9), seedlings were treated with chlorsulfuron at varying rates. All herbicides were applied with 0.2% v/v BS1000 non-ionic surfactant (Wetter TX® NuFarm, South Australia). Herbicide treatments were applied in a laboratory cabinet (Plate 3.1) through two Fan Jet Nozzles F-110-01 (Hardi, Australia) placed 40 cm above the plants in a water volume of 110 L ha^{-1} at a pressure of 250 kPa. A moving belt carried the nozzles at a speed of 1 ms^{-1} Control plants were treated with water and 0.2% v/v BS1000 non-ionic surfactant. After treatments plants were returned to the glasshouse. Thirty days after treatment each plant was recorded as dead or alive. Plant harvested for dry matter were cut at soil surface and dried at 80°C for 48 hours.

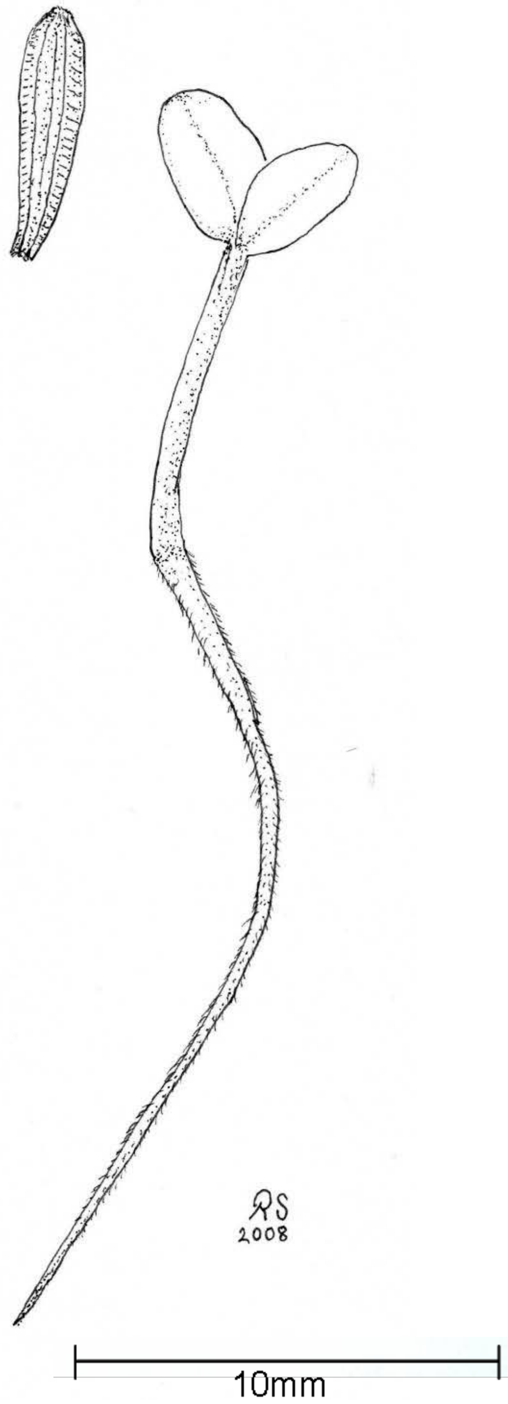

Figure 3.8 *S. oleraceus* seed with a seedling 3 days after germination.

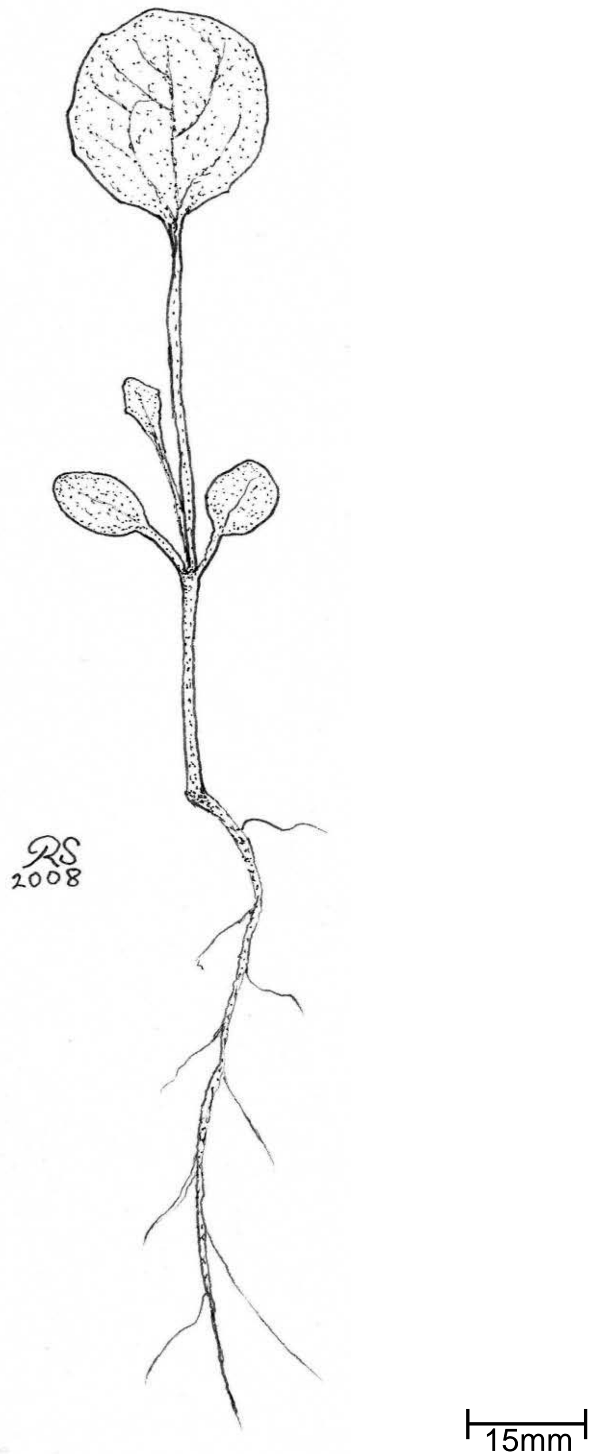

Figure 3.9 Two leaf *S. oleraceus* seedling 21 days after germination. Note two cotyledon leaves, first unifoliate leaf and second true leaf emerging.

Plate 3.1 *S. oleraceus* plants after treatment in spray cabinet, the boom with two yellow nozzles can be seen at the end of the spray run.

3.4 MOLECULAR METHODS

3.4.1 DNA EXTRACTION

3.4.1.1 CTAB extraction

CTAB DNA extraction was used for preliminary primer set screening as in section 3.4.2. Subsequent to this QUIAGEN DNeasy DNA extraction was used as described in section 3.4.1.2. DNA was extracted from the collected 1 cm^2 samples of leaf material by the following method. To each 1.5 mL eppendorf tube containing the leaf sample, 8 µL of 0.2% (v/v) ß-mercaptoethanol was added to 4 mL of cetyltrimethylammonium bromide (CTAB) isolation buffer (2% CTAB in 0.1 M Tris pH 8.0, 0.05 M ethylenediaminetetra acetic acid di-sodium salt pH 8.0, 0.5 M sodium chloride, 1% polyvinyl pyrrolidone (vinylpyrrolidine homopolymer) Mw 40,000) in a 10 mL centrifuge tube. The tube was then capped, gently inverted to mix the mercaptoethanol and isolation buffer and then placed in a water bath at

60°C for 2 hrs. Isolation buffer (500 µL) was added to the plant tissue and the tissue macerated with a sterile micro pestle till homogenised. The homogenised tissue and buffer was incubated at 60°C for 90 minutes with occasional swirling. Chloroform (500 µL) was added to the vial and gently inverted for 60 seconds prior to centrifuging in an Eppendorf 5417 C tabletop centrifuge (Eppendorf, Hamburg, Germany) for 4 minutes at 20,000 *g* (14,000 rpm). A portion of the supernatant (0.45 mL) was then transferred to a new 1.5 mL tube and 0.5 volume of 5M NaCl (0.225 mL) added. Isopropanol (0.45 mL) was then added to the tube and gently mixed to precipitate out the nucleic acids. The tube was spun for 5 minutes at 20,000 *g*, leaving a beige speck at the base of the centrifuge tube. The supernatant was carefully removed with a pipette leaving the pellet at the base of the tube. Wash buffer of 76% (v/v) ethanol and 10 mM ammonium acetate (0.5 mL) was added. The tube was gently inverted to help wash the pellet and then set aside for 20 minutes. The wash buffer was carefully removed with a clean pipette. This process was repeated twice. On the last wash the tube was then centrifuged at 20,000 *g* for 2 minutes before the liquid was removed leaving the pellet behind. A sterile cotton bud was used to remove the last remaining traces of liquid from around the pellet. The pellet was then resuspended with 50 µL of TE (10 mM Tris EDTA) buffer (pH 7.4 to 8.0) (Appendix 6) taking care not to shear or break the DNA. 4.5 µL of 4 mg/mL RNase solution was added to the tube, which was then incubated at 37°C for 30 minutes. The DNA solution was then stored at -20°C until required.

3.4.1.2 *QIAGEN DNeasy extraction* of DNA

DNA extraction was also performed using a QIAGEN® DNeasy Plant Mini kit. Steps were performed at room temperature (15°C-25°C). 1.5 mL eppendorf tubes (usually in groups of 10) were lined up in a lab rack with each tube containing a fresh 1 cm^2 piece of leaf tissue. 400 µL of buffer AP1 and 4 µL of RNase A were added to each tube and the tissue disrupted with a plastic pestle and a few sterile fine sand grains. After disruption, the tubes were sealed

and placed in a 65°C water bath for ten minutes to incubate. Tubes were inverted three times during incubation. After ten minutes 130 µL of buffer AP2 was added, gently mixed and incubated on ice for 5 minutes to precipitate detergents, proteins and polysaccharides. The tubes containing the lysates were then centrifuged for 5 minutes at 20,000 *g* (14,000 RPM) to remove the precipitates. After centrifugation the supernatant (typically 500 µL) was transferred to a QIA shredder mini spin column placed in a 2 mL collection tube and centrifuged for 2 minutes at 20,000 *g*. The top of the QIA shredder mini spin column was discarded and the liquid (typically 450 µL) in the 2 mL collection tube transferred into a new 1.5 mL eppendorf tube. To the solution 675 µL of AP3 buffer was added and mixed immediately. A volume of 650 µL was transferred to the top of the mini spin column and centrifuged at 11,430 *g* (8000 rpm) for 1 minute. The flow through liquid was discarded and the remaining 450 µL transferred to the top of the column and centrifuged at 11,430 *g* for 1 minute. The 2 mL collection tube and flow through was discarded and the top of the mini spin column transferred to a new 2 mL collection tube. 500 µL of Buffer AW was transferred to the top of the mini spin column and centrifuge at 11,430 *g* for 1 minute. The flow through liquid was discarded and a second 500 µL of Buffer AW added to the mini spin column and centrifuged at 20,000 *g* for 2 minutes. The flow through base liquid was discarded and the top of the column transferred to a new 1.5 mL eppendorf tube. 80 µL of Buffer AE was added directly onto the DNeasy membrane in the mini spin column and incubated at room temperature (15° to 25°C) for 5 minutes. This was then centrifuged at 11,430 *g* for 1 minute. The minispin column was then discarded and the 1.5 mL eppendorf tube closed and kept on ice while concentration tests were performed. The concentration of DNA in the extracted samples was measured using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) at 260nm before being labelled and stored at -20°C until required.

3.4.2 POLYMERASE CHAIN REACTION

3.4.2.1 INTER SEQUENCE REPEATS PRIMER SETS

Primers complementary to inter sequence repeats, with variable three-base anchors at the 5' end and random primers were screened in PCR analyses to select the most informative primers in terms of number of bands and polymorphisms. A set of 12 primers were screened (Table 3.1). This method was not proceeded with due to superior results found when using AFLPs (section 3.4.3).

Table 3.1 Set of primers screened with *S. oleraceus* DNA.

#	Primer	Primer sequence
1	888	BDB CA CA CA CA CA CA CA
2	891	HVH TG TG TG TG TG TG TG
3	880	HVH GT GT GT GT GT GT GT
4	811	GAG AGA GAG AGA GAG AC
5	820	GTG TGT GTG TGT GTG TC
6	818	CAC ACA CAC ACA CAC AG
7	889	DBD AC AC AC AC AC AC AC
8	1423	HVH TGT TGT TGT TGT TGT
9	OPT 16	GGT GAA CGC T
10	OPT 18	GAT GCC AGA C
11	OPT 4	CAC AGA GGG A
12	OPT 5	GGG TTT GGC A

3.4.2.2 AMPLIFICATION REACTION

The amplification reactions were performed in 20 µL volumes containing 2 µL of DNA of known concentration, 13.7 µL of nanopure water, 2 µL of buffer [10 mM Tris-HCl (pH 8.8 at 25°C), 50 mM KCl, 0.1 % Triton X-100], 1.2 µL of 25 mM $MgCl_2$, 0.5 µL of 10 mM dNTPs, 0.4 µL of 0.25 µM primer and 0.2 µL of Taq (5 U/µL) DNA polymerase (Promega, Australia). A negative control without DNA template was included in each amplification reaction. The mixture was gently agitated and transferred to a Programmable Thermal Controller (PTC-100 ™, MJ Research, Inc). The machine was programmed with the following thermal profile: 2 minutes for denaturation at 94° C, followed by 35 cycles of: 30 seconds of denaturation at 94° C, 30 seconds for annealing at 54° C and 1 minute for extension

at 72° C. At the end of 35 cycles a final extension was applied at 72° C for 2 minutes. The reactions were then held at 4° C until used.

3.4.2.3 PCR PRODUCT VISUALISATION

Amplified DNA was separated on 1.5% agarose gel (GibcoBRL Ultrapure, Cat. # 15510-021) prepared in a 1 x tris-acetate-EDTA (TAE) buffer. Six µL of each amplified product was mixed with 1 µL of blue/orange loading buffer (Promega G1881) and loaded into each well. A high molecular mass standard ladder (Invitrogen Life Technologies, Carlsbad, CA, USA) was run in one well to quantify the concentration of DNA in each sample. Gel running conditions were 80 volts D.C. for 45 minutes. On completion of electrophoresis, gels were removed from the TAE buffer, drained and stained in 1 µg mL^{-1} ethidium bromide (Invitrogen Life Technologies, Carlsbad, CA, USA) in TAE running buffer for 15 minutes. Gels were then drained and washed in reverse osmosis water, then visualised under Ultra Violet light.

3.4.3 AMPLIFIED FRAGMENT LENGTH POLYMORPHISMS (AFLP)

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). The AFLP technique described by Vos *et al*., (1995) was used with the following modifications: the DNA was cut using M*se*1 and P*st*1 restriction enzymes, consequently M*se*1 and P*st*1 adapters were used in the ligation step. Concentrations of nucleotides throughout were reduced to reflect the sensitivity of a fluorescent based technique compared to the original method outlined by Vos *et al*., (1995). Pre-amplification PCR used P*st*1+A and M*se*1+C with the selective PCR amplification using dimers of P*st*1+A and M*se*1+C sequences (M*se*1+CC and P*st*1+AC: M*se*1+CT and P*st*1+AG). The P*st*1+AC and P*st*1+AG dimer primers were fluorescently labelled (Figure 3.10).

PCR products were run on an Applied Biosystems 3730, fluorescence-based DNA analyzer at the Australian Genome Research Facility (AGRF) in Adelaide. All reactions were performed in 96 well PCR polypropylene plates (Labcon, Petaluma, U.S.A.). After the addition of all reagents the well plate was sealed with sealex-PP adhesive film (Adelab, Adelaide, South Australia). Plates were then transferred to a Programmable Thermal Controller (Gradient Mastercycler, Eppendorf, Germany) or incubator for temperature variation processing. Products were stored at -20°C until required.

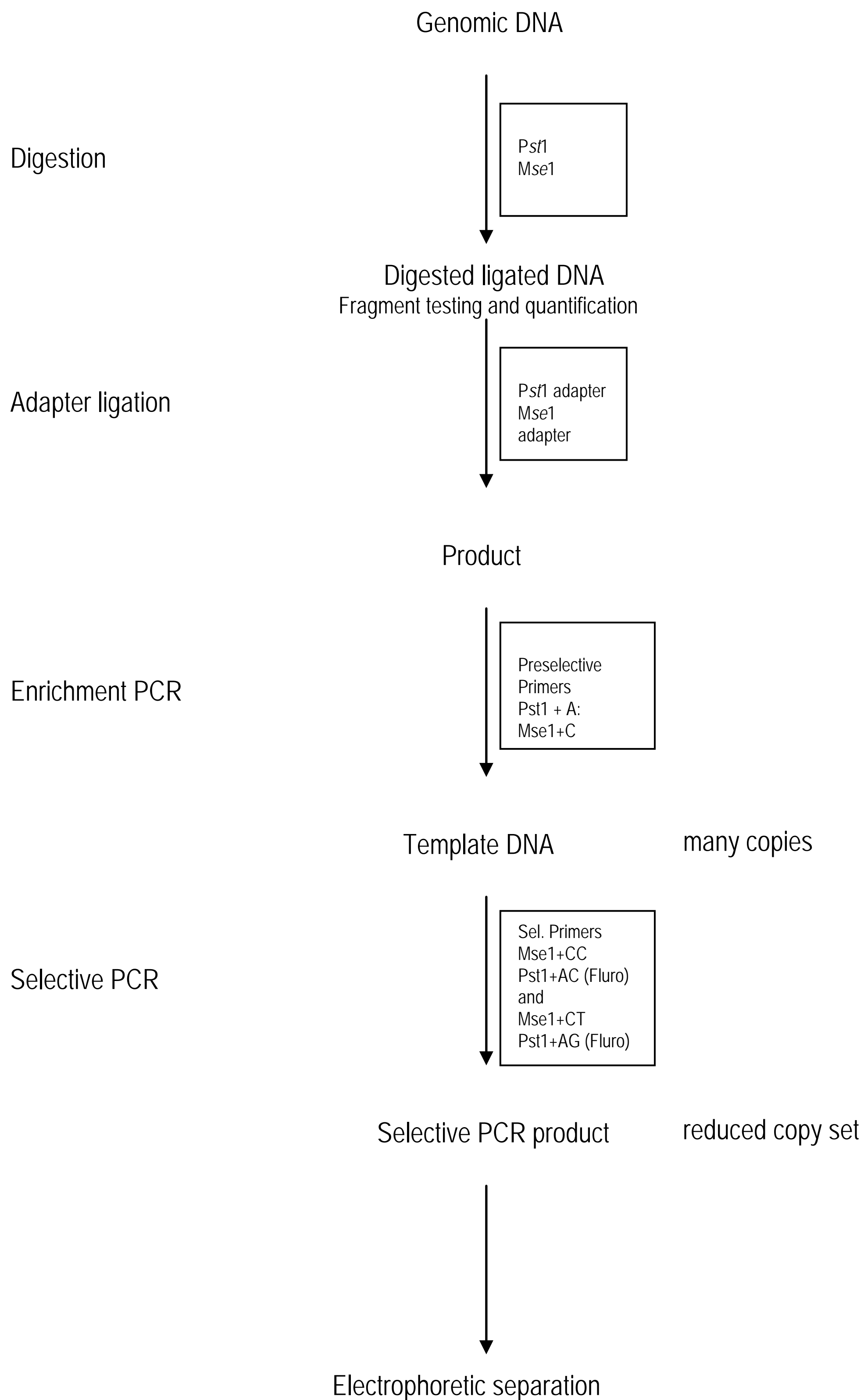

Figure 3.10 Flow chart showing the generation and detection of AFLPs from the digestion of genomic DNA with the restriction enzymes P*st1* and M*se*1.

3.4.3.1 DNA SELECTION AND ANNEALING OF ADAPTERS

The DNA of *S. oleraceus* biotypes for analysis was selected and their concentrations adjusted to a final concentration of around 150 ng/µL. M*se*I and P*st*I adapters were annealed by the method outlined in Appendix 5B and 5C. Stock solutions were prepared containing both M*se*1 adapters (Table 3.2) at 50 µL each and P*st*1 adapters (Table 3.2) at 5 µM. Stocks were heated at 90°C for 3 minutes and then come to room temperature for 30 minutes.

Table 3.2 Sequences of adapters used in modified AFLP analysis.

Oligonucleotide		
M*se*1 adapter	M-adap1	5' – GAC GAT GAG TCC TGA G – 3'
M*se*1 adapter	M-adap2	5' – TAC TCA GGA CTC AT – 3'
P*st*1 adapter	P-adap1	5' – CTC GTA GAC TGC GTA CAT GCA – 3'
P*st*1 adapter	P-adap2	5' – TGT ACG CAG TCT AC – 3'

3.4.3.2 RESTRICTION LIGATION OF DNA

The amplification reactions were performed in a 60 µL volumes in a 96 well plate. 20 µL of isolated genomic DNA with a concentration of 120 ng was transferred to the 96 well plate and kept on ice. A new 2 mL tube containing a master mix of 28.3 µL nanopure water, 6.0 µL of buffer [100 mM Tris-HCl (pH 7.5 at 25°C), 100 mM MgAcetate, 500 mMKAcetate & 50 mMDTT] two restriction enzymes P*st*1 (10 units) and M*se*1 (5 units), 0.2 mM ATP Cofactor, 0.08 µM Pst1 and 0.83 µM Mse1 adapters, and 1 Unit of T4 DNA ligase. The isolated genomic DNA was then double digested and ligated to the restricted fragments with 40 µL of master mix in the 96 well plate incubated for 3 hrs at 37° C.

Fragment testing and quantification

The cut DNA fragments were separated on 1% TAE agarose gels. Samples of 5 µL of cut DNA were loaded with 1 µL of 6X loading buffer (10% glycerol, 2 mM EDTA, 0.1% xylene cyanol and 0.1% bromophenol blue). A negative control of 5 µL nanopure water and 1 µL of

6X loading buffer was run in one cell. DNA fragment sizes were estimated by comparison to fragments present in a molecular weight marker mix 2 µL of high DNA mass ladder (Invitrogen Cat.# 10496-016) and 1 µL of 6X loading buffer. A low molecular mass ladder was used in the sequencing steps in 6.3.3. The gel was run at 80 volts for 45 minutes and then stained in ethidium bromide (1 mg/µL) for 12 minutes. The gel was gently washed with distilled water then visualised and photographed under 260 nm UV light.

3.4.3.3 PREAMPLIFICATION OF DNA

The amplification reactions were performed in a 185 µL volumes in a 96 well plate. Digested ligated DNA (5.3 µL) with a concentration of 10.6 ng was transferred to a new 96 well plate and kept on ice. A new 2 mL tube containing a master mix of 9.5 µL nanopure water, 6.0 µL of buffer (10 x Taq buffer Mg free), 1. 9 mM $MgCl_2$, 0.254 mM dNTPs, 75 ng P*st*1 primer (5' – GAC TGC GTA CAT GCA GA – 3' 5' – GAT GAG TCC TGA GTA AC – 3' with an additional A at the 3' extreme acting as the basis for selection) (Table 3.3), 75 ng M*se*1 primer (5' – GAT GAG TCC TGA GTA AC – 3' with an additional C at the 3' extreme acting as the basis for selection) (Table 3.3), and 1 Unit T*aq* polymerase.

Table 3.3 Sequences of primers used in modified AFLP analysis.

M*se*1 pre-selective primer	M+1primerC	5' – GAT GAG TCC TGA GTA AC – 3'
P*st*1 pre-selective primer	P+1primerA	5' – GAC TGC GTA CAT GCA GA – 3'
M*se*1 selective primer CC	M1 primer2	5' – GAT GAG TCC TGA GTA CC – 3'
P*st*1 selective primer AC Fluro	P1 primer1	5' – 7GA CTG CGT ACA TGC AGA C – 3'
M*se*1 selective primer CT	M2 primer2	5' – GAT GAG TCC TGA GTA – 3'
P*st*1 selective primer AG Fluro	P2 primer1	5' – 5GA CTG CGT ACA TCC AGA G – 3'

The mixture was gently agitated and transferred to a Programmable Thermal Controller (PTC-100 ™, MJ Research, Inc). The machine was programmed with the following thermal profile: 30 seconds of denaturation at 94° C, 1 minute for annealing at 54° C and 1 minute for

extension at 72° C. This was followed by a further 25 cycles of further denaturation, annealing and extension. At the end of 25 cycles a final extension was applied at 72° C for 4 minutes. The reactions were then held at 4° C for further processing.

After pre amplification in the thermocycler 160 µL of nanopure water was added to each reaction well and the plate and shaken at a slow speed for 15 minutes. Pre amplification preparations (template DNA) were stored at -20°C for further processing.

3.4.3.4 SELECTIVE PCR

The amplification reactions were performed in a 20 µL volumes in a 96 well plate. Template DNA (5.3 µL) with a concentration of 0.31 ng was transferred to a new 96 well plate and maintained on ice. This step was duplicated to accept two different master mixes containing different primers. Two new 1.5 mL tubes were used for two master mixes 1 and 2. Master mix 1 consisted of 6.65 µL nanopure water, 2.0 µL of buffer (10 x Taq buffer Mg free), 2.04 mM $MgCl_2$, 0.27 mM dNTPs, 25 ng P*st*1 fluorescent primer 1 AC (5' – 7GA CTG CGT ACA TGC AGA C – 3'), 25 ng M*se*1 primer 2 CC (5' – GAT GAG TCC TGA GTA CC – 3'), and 0.25 Units of T*aq* polymerase. Master mix 2 consisted of 6.65 µL nanopure water, 2.0 µL of buffer (10X Taq buffer Mg free), 2.04 mM $MgCl_2$, 0.27 mM dNTPs, 25 ng P*st*1 fluorescent primer 1 AG (5' – 5GA CTG CGT ACA TCC AGA G – 3') (Table 6), 25 ng M*se*1 primer 2 CT (5' – GAT GAG TCC TGA GTA – 3') (Table 3.3), and 0.25 Unit T*aq* polymerase. In the 96 well plate one group was mixed with 14.7 µL master mix 1 primer set and the other with master mix 2 primer set.

The following thermal profile was initiated: 30 seconds denaturation at 94° C, 30 seconds annealing at 65° C and 1 minute extension at 72° C, followed by 9 cycles of further denaturation, annealing and extension in which each annealing cycle was reduced by 1°C. At

the end of this sequence a second cycle of denaturation for 30 seconds at 94° C, annealing 30 seconds at 56° C and extension 1 minute at 72° C for 25 cycles was initiated. The PCR product was held in the dark at 4° C for further processing.

3.4.3.5 SEQUENCE ANALYSIS

Selective PCR product (5.3 µL) (3.4.3.4) was transferred to a new 96 well PCR polypropylene plate with 64.7 µL of nanopure water in each receiving well. This transfer was carried out in two separate groups, one for each of the two master mixes (Figure 3.11). After addition of all reagents the plate was sealed with Sealex-PP adhesive film wrapped in alfoil and delivered to the Australian Genome Research Facility for capillary analysis on an Applied Biosystems 3730, fluorescence–based DNA analyser.

	1	2	3	4	5	6	7	8	9	10	11	12
A	DNA - 1 CC-ACflur	DNA - 2 CC-ACflur	DNA - 3 CC-ACflur	DNA - 4 CC-ACflur	DNA - 5 CC-ACflur	DNA - 6 CC-ACflur	DNA - 7 CC-ACflur	DNA - 8 CC-ACflur	DNA - 9 CC-ACflur	DNA - 10 CC-ACflur	DNA - 11 CC-ACflur	DNA - 12 CC-ACflur
B	DNA - 13 CC-ACflur	DNA - 14 CC-ACflur	DNA - 15 CC-ACflur	DNA - 16 CC-ACflur	DNA - 17 CC-ACflur	DNA - 18 CC-ACflur	DNA - 19 CC-ACflur	DNA - 20 CC-ACflur	DNA - 21 CC-ACflur	DNA - 22 CC-ACflur	DNA - 23 CC-ACflur	DNA - 24 CC-ACflur
C	DNA - 25 CC-ACflur	DNA - 26 CC-ACflur	DNA - 27 CC-ACflur	DNA - 28 CC-ACflur	DNA - 29 CC-ACflur	DNA - 30 CC-ACflur	DNA - 31 CC-ACflur	DNA - 32 CC-ACflur	DNA - 33 CC-ACflur	DNA - 34 CC-ACflur	DNA - 35 CC-ACflur	DNA - 36 CC-ACflur
D	DNA - 37 CC-ACflur	DNA - 38 CC-ACflur	DNA - 39 CC-ACflur	DNA - 40 CC-ACflur	DNA - 41 CC-ACflur	DNA - 42 CC-ACflur	DNA - 43 CC-ACflur	DNA - 44 CC-ACflur	DNA - 45 CC-ACflur	DNA - 46 CC-ACflur	DNA - 47 CC-ACflur	DNA - 48 CC-ACflur
E	DNA - 1 CT-AGflur	DNA - 2 CT-AGflur	DNA - 3 CT-AGflur	DNA - 4 CT-AGflur	DNA - 5 CT-AGflur	DNA - 6 CT-AGflur	DNA - 7 CT-AGflur	DNA - 8 CT-AGflur	DNA - 9 CT-AGflur	DNA - 10 CT-AGflur	DNA - 11 CT-AGflur	DNA - 12 CT-AGflur
F	DNA - 13 CT-AGflur	DNA - 14 CT-AGflur	DNA - 15 CT-AGflur	DNA - 16 CT-AGflur	DNA - 17 CT-AGflur	DNA - 18 CT-AGflur	DNA - 19 CT-AGflur	DNA - 20 CT-AGflur	DNA - 21 CT-AGflur	DNA - 22 CT-AGflur	DNA - 23 CT-AGflur	DNA - 24 CT-AGflur
G	DNA - 25 CT-AGflur	DNA - 26 CT-AGflur	DNA - 27 CT-AGflur	DNA - 28 CT-AGflur	DNA - 29 CT-AGflur	DNA - 30 CT-AGflur	DNA - 31 CT-AGflur	DNA - 32 CT-AGflur	DNA - 33 CT-AGflur	DNA - 34 CT-AGflur	DNA - 35 CT-AGflur	DNA - 36 CT-AGflur
H	DNA - 37 CT-AGflur	DNA - 38 CT-AGflur	DNA - 39 CT-AGflur	DNA - B CT-AGf40	DNA - 41 CT-AGflur	DNA - 42 CT-AGflur	DNA - 43 CT-AGflur	DNA - 44 CT-AGflur	DNA - 45 CT-AGflur	DNA - 46 CT-AGflur	DNA - 47 CT-AGflur	DNA - 48 CT-AGflur

Figure 3.11 Ninety six well plate for capillary analysis on an Applied Biosystems 3730 fluorescence based DNA analyser. Final volume 70 µL process of 5.3 µL selective PCR product plus 64.7 µL of nanopure water. This plate has 48 duplicated DNA samples with 2 master mixes (CC-AC and CT-AG).

3.4.3.6 GENOMIC DATA ANALYSIS

Genomic data was viewed with Genemapper® software for the presence and absence of peaks at each loci. Judgements were made on peak profiles at each loci and a binary score of one for a peak (cut off 200 relative fluorescence units) and zero for no peak (less than 200 relative

fluorescence units) was assigned for each sample at each loci. A total of x loci (about 200) were scored for selective primer pair M*se*1+CC and P*st*1+AC and a total of y loci (about 200) were scored for selective primer pair M*se*1+CT and P*st*1+AG, giving a maximum of x+y loci (about 400). Data for each sample were stored on an excel file prior to transfer to a text file for analysis with PopGene® software to determine genetic relationships. Popgene® is freeware for analysis of genetic variation among and within populations using co-dominant and dominant markers, such as AFLP's. The software generated dendrograms for each population analysis, based on Nei's regular and unbiased genetic distance measures. It was used to compute genetic distance.

CHAPTER 4

4 ALS-INHIBITING HERBICIDE RESISTANCE

4.1 INTRODUCTION

There are 4 chemical families within the ALS-inhibiting herbicides that have been commercialised in Australia. These are the sulfonylureas, imidazolinones, sulfonamides and the pyrimidinylthiobenzoates. Resistance to each of these families are known and different mutations can result in different patterns of resistance (Tranel and Wright 2002). The first ALS inhibiting herbicide to be used in Australia was chlorsulfuron (a sulfonylurea), which was widely used in the cereal growing regions in the early 1980s (Boutsalis 1996). Since the discovery of the first biotype of *S. oleraceus* resistant to ALS inhibiting herbicides in Australia (Boutsalis and Powles 1995a), others have confirmed additional populations of *S. oleraceus* with resistance to ALS-inhibiting herbicides in Queensland and New South Wales (Adkins *et al*., 1997, Widderick 2002 and Walker *et al.,* 2005b).

As previously reviewed in 2.3.2.5, cross resistance to a number of different herbicides in the same group (for example sulfonylureas) can be found to exist in a single plant accession. However, the patterns of resistance produced by a dose rate experiment can vary. Acknowledging that exceptions exist, resistance caused by altered ALS can be classified into 3 groups of cross resistance: 1. Sulfonylurea and Sulfonamides; 2. Imidazolinones and Pyrimidinylthiobenzoates; and 3. Sulfonylurea, Sulfonamides, Imidazolinones and Pyrimidinylthiobenzoates (Tranel and Wright 2002).

Since it is known that in the last two decades, numerous populations of *S. oleraceus* in southern Queensland have evolved resistance to ALS-inhibiting herbicides (Widderick 2002), it follows that this resistance may be evolving in other locations in Australia. To ascertain the extent of ALS-inhibiting herbicide resistant *S. oleraceus* in Australia, seed was collected (with

GPS locations) and tested to determine whether resistance exists or not. In 2005 the first seed collection from *S. oleraceus* plants was from a bulk of approximately 10 plants in a field at Roseworthy, South Australia. On treatment with a sulfonylurea herbicide it was discovered that there was a mixture of susceptible and resistant plants. Since *S. oleraceus* is self pollinated (Barber 1941), it was suspected that individual plants either did or did not have the mutation for resistance. However, some rare cross pollination has been reported (Stebbins *et al.*, 1953; Lewin (1975) and Boutsalis and Powles (1995b). Preliminary work found that seed from individual plants produced either 100% resistant or susceptible progeny.

All seed in the collection from 2005 to 2008 were from individual single plants (accessions). From this seed, resistance screening was conducted. In section 4.2 the sensitivity of *S. oleraceus* germplasm collected from across Australia to chlorsulfuron was tested. In addition, the distribution of resistance in *S. oleraceus* across Australia is tabulated. From these results populations were selected for studies to understand the spread of ALS-inhibitor resistance in this species.

Since resistance to sulfonylureas and imidazolinones is known to be caused by different mutations (Sathasivan *et al.*, 1991) there is a need to screen some sulfonylurea resistant populations against imidazolinones to ascertain whether the resistant *S. oleraceus* plants are cross resistant to these herbicides. In section 4.3 the treatment of 22 *S. oleraceus* lines (resistant to chlorsulfuron) with an imidazolinone herbicide (imazethapyr) were assessed.

Dose rate experiments are conducted to determine the plants sensitivity when treated with a herbicide (White 2002) and they are an important tool in weed science (Seefeldt *et al.*, 1995). Here I report on the sensitivity of 9 *S. oleraceus* biotypes to chlorsulfuron to test the

hypothesis that *S. oleraceus* populations throughout Australia vary in their sensitivity to different dose rates of chlorsulfuron.

4.2 MATERIALS AND METHODS

4.2.1 *S. oleraceus* seed collection

A collection of seed was compiled from past collections maintained at the Waite Institute since 1991, Queensland Primary Industry and Fisheries (1997-1998) and recent collections between 2005 and 2008 were from southern Queensland, northern New South Wales, South Australia, Western Australia, Victoria and Tasmania. Seed was collected from single plant accessions. Dry seed was stored in labelled envelopes placed in sealed boxes and refrigerated at 3°C until required for further tests.

4.2.2 Seed and seedling treatment - chlorsulfuron

Seeds from each of the accessions were germinated on 0.6% (w/v) agar as section 3.3. After 7 days seedlings were transplanted to 22 x 9 individual cell trays containing pasteurised commercial premium potting mix with 9 seedlings planted on a column per accession. At the two to three leaf stage 28 days after germination the trays were treated with chlorsulfuron at 15g a.i. ha^{-1} + 0.2% (v/v) non-ionic surfactant. Herbicide treatment was applied in a laboratory cabinet sprayer as section 3.3. After treatment labelled trays were held in the spray treatment laboratory for one day to allow the spray treatment to dry before being allocated to space in the glasshouse. Plants were watered as required to maintain the soil at field capacity.

4.2.3 Seed and seedling treatment - imidazolinone

Seed for imidazolinone (imazethapyr) screening was selected from 15 lines resistant to the chlorsulfuron from field south 4 at Roseworthy, South Australia (Table 4.3.2.4). Seed and seedling treatment is as 3.3 but at the two to three leaf stage 28 days after germination the trays were treated with imazethapyr at 75g a.i. ha^{-1} + 0.2% (v/v) non-ionic surfactant.

4.2.4 Seed and seedling treatment – dose rate experiment

4.2.4.1 Plant material selected for treatment

From preliminary experiments using a single chlorsulfuron rate of 15 g a. i. ha^{-1} conducted in late 2005 and early 2006 with *S. oleraceus* biotypes, five resistant and four susceptible populations (Table 4.1) were selected for dose response experiments. Accessions were randomly selected with approximately half resistant and half susceptible.

Table 4.1 Geographic location of *S.oleraceus* plants selected for inclusion in the dose rate experiment and their response to chlorsulfuron 15 g a. i. ha^{-1} (20 g ha^{-1} Glean®).

Sonchus number	Location	Latitude/Longitude		Response to chlorsulfuron [a.]
So 56	Paradise, South Australia	34° 52' 24.81" S	138° 40' 08.01" E	Susceptible
So 57	[b.]Goondiwindi, Queensland	28° 32' 49.33" S	150° 18' 26.66" E	Resistant
So 101	Wasleys, South Australia	34° 28' 51.65" S	138° 41' 12.81" E	Resistant
So 102	Roseworthy, South Australia	34° 29' 52.75" S	138° 41' 18.12" E	Resistant
So 103A	Nairne, South Australia	35° 02' 42.44" S	138° 54' 43.40" E	Susceptible
So 103B	Nairne, South Australia	35° 02' 42.44" S	138° 54' 43.40" E	Susceptible
So 125	Dalby, Queensland	27° 08' 93.00" S	151° 17' 11.00" E	Resistant
So 220	Wasleys, South Australia	34° 28' 41.38" S	138° 40' 51.99" E	Resistant
So 234	Roseworthy, South Australia	34° 30' 10.20" S	138° 41' 24.22" E	Susceptible

[a] Response to chlorsulfuron - resistant:- 100% survival and no biomass reduction, susceptible: 0% survival and biomass elimination

[b] Goondiwindi, Queensland. First ALS-inhibiting herbicide resistant plant found in Australia in 1991.

4.2.4.2 Treatment methodology

The experiment was conducted in a glasshouse at the University of Adelaide, Waite Campus in South Australia. It was a randomised complete block design with 4 replicates where 9 factor biotypes (So56, So57, So101, So102, So103A, So103B, So125, So220 and So234)

were randomly assigned to the experiment and the 8 treatments (0, 1.875 g, 3.75 g, 7.5 g, 15 g, 30 g, 60 g and 120 g ha^{-1} chlorsulfuron) were also randomly allocated. On 24 March 2006 120 seeds from each of the 9 biotypes were sown on 0.6% agar in individual containers. The seeds were germinated and treated as general methods section 3.3. On 3 April 2006, 32 even seedlings (8 treatments by 4 replicates) were transplanted to 12 x 12 cm pots containing pasteurised, commercial, premium potting mix (3.3.1). All pots were labelled and randomly allocated in the glasshouse. At the two to three-leaf stage, on the 21 April 2006, 4 replicates of each of the 9 biotypes were separated into 8 treatment groups. One group of 32 plants was not treated with chlorsulfuron and retained as a control. The seven remaining groups were treated with 1.875 g, 3.75 g, 7.5 g, 15 g, 30 g, 60 g and 120 g ha^{-1} chlorsulfuron + 0.2% (v/v) non-ionic surfactant. Herbicide treatments were applied in a laboratory cabinet sprayer (as section 3.3.5). After treatment plants were returned to the glasshouse. Plants were watered as required to field capacity until the end of the experiment.

4.2.4.3 Measurements

To assess the response of individual plants to chlorsulfuron, dry matter biomass was measured and an assessment of plant status (dead or alive) was made 30 days after treatment. When assessing dry matter biomass, plants were carefully cut at soil level, ensuring all above ground plant material was sampled. Plant material was dried at $80^{o}C$ for 2 days prior to weighing.

4.2.4.4 Data analysis

Statistical analysis of the dose response curves followed the procedure detailed by Seefeldt *et al.* (1995). Data were fitted to the log-logistic model: $Y = C + (D - C)/(1 + \exp(b(\text{In}(x) – \text{In}(GR_{50}))))$ where Y = shoot weight (% of untreated control), x = herbicide dose (g ha^{-1}; a value of 1.0 was added to each dose to calculate natural logarithms, In), C = lower limit of the response curve, D = upper limit, b = slope and GR_{50} = dose (g ha^{-1}) of herbicide that reduced

shoot weight by 50% relative to the untreated control. Data were fitted to the model using Prism 5 ®.

4.3 RESULTS AND DISCUSSION

The results of this experiment (Tables 4.2 and 4.7) indicate a varying frequency in the level of resistance between individual states in Australia and that resistance to chlorsulfuron is now widespread in Australia as summarized in Table 4.8 Specific detail of the location of resistant and susceptible plants, state by state are presented in Tables 4.2 to 4.7. From these lists, plants were selected for use in the dose rate experiment, gene flow experiment and for AFLP analysis.

4.3.1 Queensland *S. oleraceus* chlorsulfuron treatment

The results from the Queensland accessions tested (Table 4.2) show varying sensitivity to chlorsulfuron with 58 accessions being resistant and 40 susceptible. The progeny from the seed of 23 accessions taken from a field at Warra produced 11 resistant and 12 susceptible populations (Table 4.2, footnote [c]). Similarly the progeny from the seed of 11 individual plants taken from a field in the Condamine produced 10 resistant and 1 susceptible accessions (Table 4.2, footnote [d]).

Table 4.2 The collection locations of *S. oleraceus* accessions from Queensland and the response of their progeny to the ALS-inhibiting herbicide, chlorsulfuron (15 g a. i. ha^{-1}).

Sonchus number	Location	Latitude/Longitude		Response to chlorsulfuron [a.]
So 1	Billa Billa, Queensland	28° 10' 00.00" S	150° 16' 00.00" E	Resistant
So 7	Billa Billa, Queensland	28° 10' 00.00" S	150° 16' 00.00" E	Susceptible
So 14	Dalby, Queensland	27° 08' 93.00" S	151° 17' 11.00" E	Resistant
So 16	Jackson, Queensland	26° 38' 29.49" S	149° 41' 51.19" E	Resistant
So 17A	Gatton, Queensland	27° 33' 34.00" S	152° 16' 46.00" E	Susceptible
So 17B	Gatton, Queensland	27° 33' 34.00" S	152° 16' 46.00" E	Susceptible
So 34	Roma, Queensland	26° 34' 51.54" S	148° 57' 27.02" E	Susceptible
So 57	[b.]Goondiwindi, Queensland	28° 32' 49.33" S	150° 18' 26.66" E	Resistant
So 123	Oakey, Queensland	27° 24' 57.00" S	151° 39' 70.00" E	Susceptible
So 124	Dalby, Queensland	27° 18' 20.00" S	151° 26' 32.00" E	Susceptible
So 125	Dalby, Queensland	27° 08' 93.00" S	151° 17' 11.00" E	Resistant
So 126	Dalby, Queensland	27° 08' 93.00" S	151° 17' 11.00" E	Susceptible
So 127	Dalby, Queensland	27° 08' 93.00" S	151° 17' 11.00" E	Susceptible
So 128	[c.]Warra, Queensland	26° 53' 82.00" S	150° 51' 08.00" E	Susceptible
So 129	[c.]Warra, Queensland	26° 53' 15.00" S	150° 51' 48.00" E	Susceptible
So 130	[c.]Warra, Queensland	26° 53' 17.00" S	150° 51' 47.00" E	Resistant
So 131	[c.]Warra, Queensland	26° 53' 18.00" S	150° 51' 45.00" E	Resistant
So 132	[c.]Warra, Queensland	26° 53' 19.00" S	150° 51' 44.00" E	Resistant
So 133	[c.]Warra, Queensland	26° 53' 20.00" S	150° 51' 43.00" E	Resistant
So 134	[c.]Warra, Queensland	26° 53' 18.00" S	150° 51' 43.00" E	Resistant
So 135	[c.]Warra, Queensland	26° 53' 16.00" S	150° 51' 44.00" E	Susceptible
So 136	[c.]Warra, Queensland	26° 53' 14.00" S	150° 51' 44.00" E	Susceptible
So 137	[c.]Warra, Queensland	26° 53' 12.00" S	150° 51' 44.00" E	Susceptible
So 138	[c.]Warra, Queensland	26° 53' 12.00" S	150° 51' 42.00" E	Susceptible
So 139	[c.]Warra, Queensland	26° 53' 13.00" S	150° 51' 42.00" E	Susceptible
So 140	[c.]Warra, Queensland	26° 53' 14.00" S	150° 51' 40.00" E	Susceptible
So 141	[c.]Warra, Queensland	26° 53' 15.00" S	150° 51' 40.00" E	Susceptible
So 142	[c.]Warra, Queensland	26° 53' 16.00" S	150° 51' 37.00" E	Susceptible
So 143	[c.]Warra, Queensland	26° 53' 14.00" S	150° 51' 37.00" E	Resistant
So 144	[c.]Warra, Queensland	26° 53' 12.00" S	150° 51' 37.00" E	Susceptible
So 145	[c.]Warra, Queensland	26° 53' 10.00" S	150° 51' 38.00" E	Resistant
So 146	[c.]Warra, Queensland	26° 53' 07.00" S	150° 51' 40.00" E	Resistant
So 147	[c.]Warra, Queensland	26° 53' 07.00" S	150° 51' 38.00" E	Resistant
So 148	[c.]Warra, Queensland	26° 53' 09.00" S	150° 51' 37.00" E	Resistant
So 149	[c.]Warra, Queensland	26° 53' 10.00" S	150° 51' 36.00" E	Resistant
So 150	[c.]Warra, Queensland	26° 53' 12.00" S	150° 51' 34.00" E	Susceptible
So 151	Condamine, Queensland	26° 55' 35.00" S	150° 04' 64.00" E	Resistant
So 152	Condamine, Queensland	27° 06' 12.00" S	149° 56' 44.00" E	Susceptible
So 153	Condamine, Queensland	27° 06' 12.00" S	149° 58' 17.00" E	Susceptible
So 154	Meandarra, Queensland	27° 22' 00.00" S	150° 04' 71.00" E	Resistant
So 155	Dalby, Queensland	27° 16' 00.00" S	150° 05' 69.00" E	Resistant
So 156	Brookstead, Queensland	27° 47' 00.00" S	151° 24' 51.00" E	Resistant
So 157	Millmeran, Queensland	28° 00' 53.00" S	150° 53' 99.00" E	Susceptible
So 158	Goondiwindi, Queensland	28° 09' 97.00" S	150° 25' 79.00" E	Susceptible
So 159	Billa Billa, Queensland	28° 10' 00.00" S	150° 27' 00.00" E	Resistant
So 160	Billa Billa, Queensland	28° 07' 61.00" S	150° 15' 29.00" E	Susceptible
So 161	Billa Billa, Queensland	28° 07' 00.00" S	150° 15' 29.00" E	Resistant
So 162	Billa Billa, Queensland	28° 05' 00.00" S	150° 15' 08.00" E	Resistant
So 163	Moonie, Queensland	27° 44' 00.00" S	150° 19' 80.00" E	Resistant
So 164	Moonie, Queensland	27° 44' 00.00" S	150° 15' 96.00" E	Resistant
So 165	Bungunya, Queensland	28° 01' 43.00" S	149° 41' 93.00" E	Susceptible
So 168	Bungunya, Queensland	28° 25' 00.00" S	149° 42' 05.00" E	Resistant

So 170	Bungunya, Queensland	28° 25' 00.00" S	149° 42' 05.00" E	Resistant
So 171	Goondiwindi, Queensland	28° 29' 00.00" S	150° 25' 08.00" E	Resistant
So 243	Roma, Queensland	26° 39' 09.48" S	148° 52' 31.54" E	Susceptible
So 244	St. George, Queensland	27° 44' 48.22" S	149° 42' 59.05" E	Susceptible
So 245	Meandarra, Queensland	27° 36' 00.00" S	149° 45' 00.00" E	Resistant
So 246	Moonie, Queensland	27° 40' 00.00" S	150° 24' 00.00" E	Resistant
So 247	Goodar, Queensland	28° 07' 00.00" S	149° 52' 00.00" E	Resistant
So 248	Boonarga, Queensland	26° 49' 14.33" S	150° 48' 36.58" E	Susceptible
So 250	Chinchilla, Queensland	26° 44' 00.00" S	150° 37' 00.00" E	Resistant
So 251	Condamine, Queensland	26° 56' 00.00" S	150° 08' 00.00" E	Resistant
So 252	Condamine, Queensland	26° 56' 00.00" S	150° 06' 00.00" E	Resistant
So 253	Emerald, Queensland	23° 51' 51.22" S	148° 17' 12.07" E	Susceptible
So 340	Warra, Queensland	26° 55' 41.00" S	150° 55' 02.00" E	Susceptible
So 341	Chinchilla, Queensland	26° 48' 00.00" S	150° 34' 37.00" E	Susceptible
So 343	[d]Condamine, Queensland	27° 06' 24.00" S	150° 08' 29.00" E	Resistant
So 344	[d]Condamine, Queensland	27° 06' 23.00" S	150° 00' 27.00" E	Resistant
So 345	[d]Condamine, Queensland	27° 06' 22.00" S	150° 08' 27.00" E	Resistant
So 346	[d]Condamine, Queensland	27° 06' 21.00" S	150° 08' 22.00" E	Resistant
So 347	[d]Condamine, Queensland	27° 06' 22.00" S	150° 08' 32.00" E	Resistant
So 348	[d]Condamine, Queensland	27° 06' 19.00" S	150° 08' 34.00" E	Resistant
So 349	[d]Condamine, Queensland	27° 06' 22.00" S	150° 08' 00.00" E	Resistant
So 350	[d]Condamine, Queensland	27° 06' 22.00" S	150° 08' 32.00" E	Resistant
So 351	[d]Condamine, Queensland	27° 06' 23.00" S	150° 00' 33.00" E	Resistant
So 352	[d]Condamine, Queensland	27° 06' 27.00" S	150° 08' 31.00" E	Susceptible
So 353	[d]Condamine, Queensland	27° 06' 29.00" S	150° 08' 00.00" E	Resistant
So 356	Brookstead, Queensland	27° 45' 30.00" S	151° 28' 56.00" E	Resistant
So 358	Brookstead, Queensland	27° 45' 31.00" S	151° 28' 54.00" E	Resistant
So 364	Millmerren, Queensland	27° 49' 51.00" S	150° 20' 00.00" E	Resistant
So 365	Brookstead, Queensland	27° 49' 52.00" S	151° 20' 56.00" E	Resistant
So 369	Goondiwindi, Queensland	28° 15' 48.00" S	150° 23' 00.00" E	Resistant
So 370	Goondiwindi, Queensland	28° 17' 54.00" S	150° 22' 19.00" E	Resistant
So 371	[e]Goondiwindi, Queensland	28° 16' 57.00" S	150° 22' 56.00" E	Resistant
So 372	[e]Goondiwindi, Queensland	28° 16' 56.00" S	150° 22' 00.00" E	Resistant
So 373	[e]Goondiwindi, Queensland	28° 16' 56.00" S	150° 22' 59.00" E	Susceptible
So 374	[e]Goondiwindi, Queensland	28° 16' 57.00" S	150° 22' 00.00" E	Resistant
So 375	[e]Goondiwindi, Queensland	28° 16' 57.00" S	150° 22' 59.00" E	Resistant
So 376	[e]Goondiwindi, Queensland	28° 16' 57.00" S	150° 23' 01.00" E	Susceptible
So 378	[e]Goondiwindi, Queensland	28° 16' 58.00" S	150° 23' 02.00" E	Susceptible
So 423	Goondiwindi, Queensland	28° 18' 00.00" S	150° 17' 00.00" E	Resistant
So 424	Goondiwindi, Queensland	28° 18' 00.00" S	150° 17' 41.00" E	Susceptible
So 425	Goondiwindi, Queensland	28° 18' 00.00" S	150° 17' 00.00" E	Resistant
So 426	Goondiwindi, Queensland	28° 11' 21.00" S	150° 27' 00.00" E	Resistant
So 427	Goondiwindi, Queensland	28° 11' 21.00" S	150° 27' 00.00" E	Resistant
So 428	[f]Toowoomba, Queensland	28° 53' 01.00" S	150° 23' 00.00" E	Resistant
So 429	[f]Toowoomba, Queensland	28° 53' 01.00" S	150° 23' 59.00" E	Susceptible
So 431	[f]Toowoomba, Queensland	27° 35' 18.00" S	151° 56' 54.00" E	Susceptible

[a] Response to chlorsulfuron - resistant:- 100% survival and no biomass reduction, susceptible: 0% survival and biomass elimination

[b] Goondiwindi, Queensland. First ALS-inhibiting herbicide resistant plant found in Australia in 1991.

[c] Warra, Queensland. Plants from a single field.

[d] Condamine, Queensland. Plants from a single field.

[e] Goondiwindi, Queensland. Plants from a single field.

[f] Toowoomba, Queensland. Plant from town centre.

4.3.2 South Australian *S. oleraceus* chlorsulfuron treatment

The results from the South Australian accessions tested (Table 4.3), show varying sensitivity to chlorsulfuron with 35 accessions being resistant and 84 susceptible. The progeny from the seed of 37 accessions taken from a single field at Roseworthy produced 15 resistant and 22 susceptible populations (Table 4.3, footnote [g]). Detailed studies of plants from this field are covered in Chapter 7. Plants from the roadside adjacent to this field proved to be a mixture of resistant and susceptible individuals. However, a collection of 9 plants from a different field on the same farm all proved to be susceptible. A single resistant plant was found at Stirling in the Adelaide Hills, where few ALS-inhibiting herbicides are used. It is possible this seed arrived by wind from elsewhere and shows the mobile nature of the wind blown seeds. At one site in metropolitan Adelaide, 4 resistant plants were found growing close together. This may have been caused by evolution of resistance from council roadside spraying over numerous years.

Table 4.3 The collection locations of *S. oleraceus* accessions from South Australia and the response of their progeny to the ALS-inhibiting herbicide, chlorsulfuron (15 g a. i. ha^{-1}).

Sonchus number	Location	Latitude/Longitude		Response to chlorsulfuron [a]
So 56	Paradise, South Australia	34° 52' 24.81" S	138° 40' 08.01" E	Susceptible
So 101	Wasleys, South Australia	34° 28' 51.65" S	138° 41' 12.81" E	Resistant
So 102	Roseworthy, South Australia	34° 29' 52.75" S	138° 41' 18.12" E	Resistant
So 103	Nairne, South Australia	35° 02' 42.44" S	138° 54' 43.40" E	Susceptible
So 104	St. Kilda, South Australia	34° 42' 54.57" S	138° 34' 35.13" E	Susceptible
So 106	Virginia, South Australia	34° 38' 56.29" S	138° 34' 21.94" E	Resistant
So 107	Roseworthy, South Australia	34° 34' 15.74" S	138° 40' 06.32" E	Susceptible
So 108	Wasleys, South Australia	34° 33' 09.35" S	138° 41' 32.88" E	Resistant
So 114	Adelaide, South Australia	34° 56' 35.35" S	138° 34' 53.29" E	Susceptible
So 115	Adelaide, South Australia	34° 56' 35.35" S	138° 34' 53.29" E	Susceptible
So 120	Urrbrae, South Australia	34° 58' 06.99" S	138° 38' 02.95" E	Susceptible
So 121	Wasleys, South Australia	34° 28' 20.95" S	138° 41' 10.44" E	Susceptible
So 122	Wasleys, South Australia	34° 28' 20.95" S	138° 41' 10.44" E	Susceptible
So 178	Adelaide, South Australia	34° 56' 05.22" S	138° 36' 45.92" E	Susceptible
So 179	Myrtle Bank, South Australia	34° 57' 44.93" S	138° 38' 17.55" E	Susceptible
So 196	Myrtle Bank, South Australia	34° 57' 44.93" S	138° 38' 17.55" E	Susceptible
So 197	Glenunga, South Australia	34° 57' 33.23" S	138° 38' 58.08" E	Resistant
So 198	Urrbrae, South Australia	34° 58' 13.55" S	138° 38' 22.89" E	Susceptible
So 199	Myrtle Bank, South Australia	34° 57' 44.80" S	138° 38' 17.60" E	Resistant
So 200	Myrtle Bank, South Australia	34° 57' 44.94" S	138° 38' 17.55" E	Susceptible
So 201	Myrtle Bank, South Australia	34° 57' 44.95" S	138° 38' 17.55" E	Susceptible
So 215	Myrtle Bank, South Australia	34° 57' 44.96" S	138° 38' 17.55" E	Susceptible
So 216	Myrtle Bank, South Australia	34° 57' 43.01" S	138° 38' 25.85" E	Susceptible
So 217	Urrbrae, South Australia	34° 58' 06.99" S	138° 38' 02.95" E	Susceptible
So 220	Wasleys, South Australia	34° 28' 41.38" S	138° 40' 51.99" E	Resistant
So 230	Eden Hills, South Australia	35° 00' 53.33" S	138° 34' 54.77" E	Susceptible
So 234	Roseworthy, South Australia	34° 30' 10.20" S	138° 41' 24.22" E	Susceptible
So 239	Port Lincoln, South Australia	34° 44' 46.01" S	135° 52' 45.74" E	Susceptible
So 240	Port Lincoln, South Australia	34° 44' 46.02" S	135° 52' 45.74" E	Susceptible
So 241	Port Lincoln, South Australia	34° 44' 46.03" S	135° 52' 45.74" E	Susceptible
So 242	Port Lincoln, South Australia	34° 44' 46.04" S	135° 52' 45.74" E	Susceptible
So 266	Roseworthy, South Australia	34° 30' 43.38" S	138° 41' 20.06" E	Susceptible
So 267	Roseworthy, South Australia	34° 30' 43.39" S	138° 41' 20.06" E	Susceptible
So 268	Roseworthy, South Australia	34° 30' 43.40" S	138° 41' 20.06" E	Susceptible
So 269	Roseworthy, South Australia	34° 32' 24.31" S	138° 41' 29.26" E	Susceptible
So 270	Wasleys, South Australia	34° 28' 40.00" S	138° 40' 51.00" E	Resistant
So 271	Wasleys, South Australia	34° 28' 16.00" S	138° 40' 41.00" E	Resistant
So 272	Gawler, South Australia	34° 34' 02.62" S	138° 43' 37.17" E	Susceptible
So 273	Roseworthy, South Australia	34° 30' 54.50" S	138° 41' 55.00" E	Resistant
So 274	Roseworthy, South Australia	34° 30' 58.11" S	138° 42' 34.10" E	Susceptible
So 275	Nairne, South Australia	35° 02' 42.54" S	138° 54' 42.98" E	Susceptible
So 276	Semaphore, South Australia	35° 50' 22.22" S	138° 28' 52.11" E	Susceptible
So 277	[h]Roseworthy, South Australia	34° 30' 47.65" S	138° 41' 22.10" E	Susceptible
So 278	[h]Roseworthy, South Australia	34° 30' 46.82" S	138° 41' 21.89" E	Susceptible
So 279	[h]Roseworthy, South Australia	34° 30' 47.72" S	138° 41' 21.70" E	Susceptible
So 280	[h]Roseworthy, South Australia	34° 30' 44.35" S	138° 41' 21.77" E	Susceptible
So 281	[h]Roseworthy, South Australia	34° 30' 43.24" S	138° 41' 21.53" E	Susceptible
So 282	[h]Roseworthy, South Australia	34° 30' 42.32" S	138° 41' 21.46" E	Susceptible
So 283	[h]Roseworthy, South Australia	34° 30' 41.04" S	138° 41' 21.87" E	Susceptible
So 284	[h]Roseworthy, South Australia	34° 30' 39.84" S	138° 41' 21.89" E	Susceptible
So 285	Baratta, South Australia	31° 56' 09.54" S	139° 05' 44.64" E	Susceptible
So 286	[g]Roseworthy, South Australia	34° 33' 05.00" S	138° 41' 32.00" E	Susceptible
So 287	[g]Roseworthy, South Australia	34° 33' 02.50" S	138° 41' 30.00" E	Susceptible

So 288	[g]Roseworthy, South Australia	34° 33' 02.50" S	138° 41' 32.50" E	Resistant
So 289	[g]Roseworthy, South Australia	34° 33' 00.00" S	138° 41' 32.50" E	Resistant
So 291	[g]Roseworthy, South Australia	34° 33' 00.00" S	138° 41' 27.50" E	Resistant
So 292	[g]Roseworthy, South Australia	34° 32' 57.50" S	138° 41' 27.50" E	Susceptible
So 293	[g]Roseworthy, South Australia	34° 32' 57.50" S	138° 41' 30.00" E	Susceptible
So 294	[g]Roseworthy, South Australia	34° 32' 57.50" S	138° 41' 32.50" E	Susceptible
So 295	[g]Roseworthy, South Australia	34° 33' 55.00" S	138° 41' 32.50" E	Resistant
So 296	[g]Roseworthy, South Australia	34° 32' 55.00" S	138° 41' 30.00" E	Susceptible
So 297	[g]Roseworthy, South Australia	34° 32' 55.00" S	138° 41' 27.50" E	Susceptible
So 298	[g]Roseworthy, South Australia	34° 32' 55.00" S	138° 41' 25.00" E	Susceptible
So 299	[g]Roseworthy, South Australia	34° 32' 52.50" S	138° 41' 22.50" E	Susceptible
So 300	[g]Roseworthy, South Australia	34° 32' 52.50" S	138° 41' 25.00" E	Susceptible
So 301	[g]Roseworthy, South Australia	34° 32' 52.50" S	138° 41' 27.50" E	Susceptible
So 302	[g]Roseworthy, South Australia	34° 33' 52.50" S	138° 41' 30.00" E	Resistant
So 303	[g]Roseworthy, South Australia	34° 32' 52.50" S	138° 41' 32.50" E	Resistant
So 304	[g]Roseworthy, South Australia	34° 33' 50.00" S	138° 41' 32.50" E	Resistant
So 305	[g]Roseworthy, South Australia	34° 32' 50.00" S	138° 41' 30.00" E	Susceptible
So 306	[g]Roseworthy, South Australia	34° 32' 50.00" S	138° 41' 27.50" E	Susceptible
So 307	[g]Roseworthy, South Australia	34° 32' 50.00" S	138° 41' 25.00" E	Susceptible
So 308	[g]Roseworthy, South Australia	34° 32' 50.00" S	138° 41' 22.50" E	Resistant
So 309	[g]Roseworthy, South Australia	34° 32' 50.00" S	138° 41' 20.00" E	Resistant
So 310	[g]Roseworthy, South Australia	34° 32' 47.50" S	138° 41' 17.50" E	Resistant
So 311	[g]Roseworthy, South Australia	34° 32' 47.50" S	138° 41' 20.00" E	Resistant
So 312	[g]Roseworthy, South Australia	34° 32' 47.50" S	138° 41' 22.50" E	Susceptible
So 313	[g]Roseworthy, South Australia	34° 32' 47.50" S	138° 41' 25.00" E	Resistant
So 314	[g]Roseworthy, South Australia	34° 32' 47.50" S	138° 41' 27.50" E	Resistant
So 315	[g]Roseworthy, South Australia	34° 32' 47.50" S	138° 41' 30.00" E	Susceptible
So 316	[g]Roseworthy, South Australia	34° 32' 45.00" S	138° 41' 30.00" E	Susceptible
So 317	[g]Roseworthy, South Australia	34° 32' 45.00" S	138° 41' 27.50" E	Susceptible
So 318	[g]Roseworthy, South Australia	34° 32' 45.00" S	138° 41' 25.00" E	Susceptible
So 319	[g]Roseworthy, South Australia	34° 32' 45.00" S	138° 41' 22.50" E	Resistant
So 320	[g]Roseworthy, South Australia	34° 32' 45.00" S	138° 41' 20.00" E	Resistant
So 321	[g]Roseworthy, South Australia	34° 32' 45.00" S	138° 41' 17.50" E	Susceptible
So 322	[g]Roseworthy, South Australia	34° 32' 45.00" S	138° 41' 15.00" E	Susceptible
So 323	Roseworthy, South Australia	34° 31' 42.00" S	138° 41' 28.00" E	Susceptible
So 324	Roseworthy, South Australia	34° 31' 38.00" S	138° 41' 35.00" E	Susceptible
So 325	Roseworthy, South Australia	34° 31' 42.00" S	138° 41' 11.00" E	Susceptible
So 326	Roseworthy, South Australia	34° 31' 42.00" S	138° 41' 11.00" E	Susceptible
So 327	Stirling, South Australia	35° 00' 14.69" S	138° 42' 54.92" E	Resistant
So 328	Stirling, South Australia	35° 00' 54.42" S	138° 42' 52.82" E	Susceptible
So 329	Roseworthy, South Australia	34° 31' 54.00" S	138° 41' 30.00" E	Susceptible
So 330	Roseworthy, South Australia	34° 31' 53.00" S	138° 41' 31.00" E	Susceptible
So 331	Roseworthy, South Australia	34° 31' 55.00" S	138° 41' 34.00" E	Susceptible
So 332	Roseworthy, South Australia	34° 31' 57.00" S	138° 41' 38.00" E	Susceptible
So 334	Roseworthy, South Australia	34° 31' 59.00" S	138° 41' 44.00" E	Susceptible
So 335	Roseworthy, South Australia	34° 31' 59.00" S	138° 41' 45.00" E	Susceptible
So 337	Roseworthy, South Australia	34° 32' 01.00" S	138° 41' 39.00" E	Susceptible
So 338	Roseworthy, South Australia	34° 32' 00.00" S	138° 41' 33.00" E	Susceptible
So 339	Roseworthy, South Australia	34° 31' 58.00" S	138° 41' 30.00" E	Susceptible
So 549	[i]Holden Hill, South Australia	34° 51' 25.92" S	138° 39' 57.79" E	Resistant
So 550	[i]Holden Hill, South Australia	34° 51' 26.00" S	138° 39' 57.79" E	Resistant
So 551	[i]Holden Hill, South Australia	34° 51' 25.18" S	138° 39' 57.79" E	Resistant
So 552	[i]Holden Hill, South Australia	34° 51' 26.31" S	138° 39' 57.79" E	Resistant
So 555	Adelaide, South Australia	34° 55' 57.52" S	138° 35' 47.11" E	Susceptible
So 556	Green Fields, South Australia	34° 47' 36.40" S	138° 35' 38.52" E	Susceptible
So 557	Green Fields, South Australia	34° 47' 36.42" S	138° 35' 38.52" E	Susceptible
So 561	[j]Roseworthy, South Australia	34° 32' 59.32" S	138° 41' 32.87" E	Susceptible
So 562	[j]Roseworthy, South Australia	34° 32' 59.28" S	138° 41' 32.87" E	Susceptible

So 564	[j]Roseworthy, South Australia	34° 32' 59.20" S	138° 41' 32.87" E	Resistant
So 565	[j]Roseworthy, South Australia	34° 32' 59.16" S	138° 41' 32.87" E	Susceptible
So 566	[j]Roseworthy, South Australia	34° 32' 55.96" S	138° 41' 32.45" E	Resistant
So 567	[j]Roseworthy, South Australia	34° 32' 55.92" S	138° 41' 32.45" E	Resistant
So 568	[j]Roseworthy, South Australia	34° 32' 55.88" S	138° 41' 32.45" E	Resistant
So 569	[j]Roseworthy, South Australia	34° 32' 55.84" S	138° 41' 32.45" E	Resistant
So 570	[j]Roseworthy, South Australia	34° 32' 55.80" S	138° 41' 32.45" E	Susceptible
So 571	Balaklava, South Australia	34° 08' 42.72" S	138° 25' 09.01" E	Susceptible

[a] Response to chlorsulfuron - resistant:- 100% survival and no biomass reduction, susceptible: 0% survival and biomass elimination.
[g] Roseworthy, South Australia. Plants from one field (S4)
[h] Roseworthy, South Australia. Plants from one field.
[i] Holden Hill, South Australia. Plants from metropolitan Adelaide street side.
[j] Roseworthy, South Australia. Plants from roadside adjoining single field South 4.

4.3.3 New South Wales *S. oleraceus* chlorsulfuron treatment

The results from the New South Wales accessions tested (Table 4.4) show varying sensitivity to chlorsulfuron with 25 accessions being resistant and 7 susceptible. The results further show varying numbers of resistant and susceptible plants from the same field. DNA from these plants was used later in the study. Seed of multiple plants were collected from 4 different fields at North Star in New South Wales. In one field six plants were collected of which three plants were resistant and three plants were susceptible. In the other three fields all plants were resistant. This highlights the need to collect at least 5 plants from any location to draw a reasonable conclusion as to the true resistance status at that location.

Table 4.4 The collection locations of *S. oleraceus* accessions from New South Wales and the response of their progeny to the ALS-inhibiting herbicide, chlorsulfuron (15 g a. i. ha^{-1}).

Sonchus number	Location	Latitude/Longitude		Response to chlorsulfuron [a]
So 5	Croppa Ck, New South Wales	27° 07' 32.00" S	150° 18' 26.00" E	Resistant
So 10	Yallaroi, New South Wales	29° 09' 00.05" S	150° 28' 20.00" E	Susceptible
So 172	North Star, New South Wales	28° 52' 76.00" S	150° 23' 96.00" E	Resistant
So 173	Yallaroi, New South Wales	28° 08' 03.00" S	150° 22' 27.00" E	Resistant
So 174	Warialda, New South Wales	29° 29' 93.00" S	150° 34' 77.00" E	Resistant
So 175	Yetman, New South Wales	28° 55' 50.00" S	150° 46' 12.00" E	Susceptible
So 176	Yetman, New South Wales	28° 55' 50.00" S	150° 46' 12.00" E	Resistant
So 249	Narabri, New South Wales	30° 00' 13.26" S	149° 38' 33.26" E	Susceptible
So 391	North Star, New South Wales	28° 48' 49.00" S	150° 25' 03.00" E	Resistant
So 397	North Star, New South Wales	28° 52' 14.00" S	150° 24' 07.00" E	Susceptible
So 401	[k.]North Star, New South Wales	28° 53' 01.00" S	150° 23' 59.00" E	Resistant
So 402	[k.]North Star, New South Wales	28° 53' 02.00" S	150° 23' 58.00" E	Resistant
So 403	[k.]North Star, New South Wales	28° 53' 03.00" S	150° 23' 58.00" E	Resistant
So 404	[k.]North Star, New South Wales	28° 53' 04.00" S	150° 23' 58.00" E	Resistant
So 405	[k.]North Star, New South Wales	28° 53' 04.00" S	150° 23' 58.00" E	Resistant
So 406	[l.]North Star, New South Wales	28° 54' 47.00" S	150° 23' 40.00" E	Resistant
So 407	[l.]North Star, New South Wales	28° 54' 48.00" S	150° 23' 40.00" E	Resistant
So 408	[l.]North Star, New South Wales	28° 54' 49.00" S	150° 23' 40.00" E	Resistant
So 409	[l.]North Star, New South Wales	28° 54' 50.00" S	150° 23' 40.00" E	Resistant
So 410	[l.]North Star, New South Wales	28° 54' 50.00" S	150° 23' 40.00" E	Resistant
So 411	[m.]North Star, New South Wales	28° 56' 36.00" S	150° 24' 03.00" E	Resistant
So 412	[m.]North Star, New South Wales	28° 56' 28.00" S	150° 24' 05.00" E	Resistant
So 413	[m.]North Star, New South Wales	28° 56' 27.00" S	150° 24' 05.00" E	Susceptible
So 414	[m.]North Star, New South Wales	28° 56' 26.00" S	150° 24' 05.00" E	Susceptible
So 415	[m.]North Star, New South Wales	28° 56' 25.00" S	150° 24' 05.00" E	Susceptible
So 416	[m.]North Star, New South Wales	28° 56' 23.00" S	150° 24' 05.00" E	Resistant
So 417	[n.]North Star, New South Wales	28° 53' 53.00" S	150° 22' 50.00" E	Resistant
So 418	[n.]North Star, New South Wales	28° 55' 54.00" S	150° 22' 49.00" E	Resistant
So 419	[n.]North Star, New South Wales	28° 55' 54.00" S	150° 22' 49.00" E	Resistant
So 420	[n.]North Star, New South Wales	28° 55' 53.00" S	150° 22' 48.00" E	Resistant
So 421	[n.]North Star, New South Wales	28° 55' 53.00" S	150° 22' 49.00" E	Resistant
So 422	[n.]North Star, New South Wales	28° 55' 53.00" S	150° 22' 49.00" E	Resistant

[a] Response to chlorsulfuron - resistant:- 100% survival and no biomass reduction, susceptible: 0% survival and biomass elimination

[k] North Star, New South Wales. Collection from close proximity in one field.

[l] North Star, New South Wales. Collection from close proximity in one field.

[m] North Star, New South Wales. Collection from close proximity in one field.

[n] North Star, New South Wales. Collection from close proximity in one field.

4.3.4 Western Australian *S. oleraceus* chlorsulfuron treatment

In Western Australian, 12 accessions were tested (Table 4.5) and showed varying sensitivity to chlorsulfuron with 2 accessions being resistant and 10 susceptible.

Table 4.5 The collection locations of *S. oleraceus* accessions from Western Australia and the response of their progeny to the ALS-inhibiting herbicide, chlorsulfuron (15 g a. i. ha^{-1}).

Sonchus number	Location	Latitude/Longitude		Response to chlorsulfuron [a]
So 505	Busselton, Western Australia	33° 39 24.40" S	115° 24' 00.00" E	Susceptible
So 506	Myalup, Western Australia	33° 06' 35.42" S	115° 42' 28.23" E	Resistant
So 509	Busselton, Western Australia	33° 39' 24.40" S	115° 24' 42.32" E	Resistant
So 516	Cowaramup, Western Australia	33° 48' 24.40" S	115° 07' 00.00" E	Susceptible
So 523	Witchcliffe, Western Australia	34° 01' 21.14" S	115° 05' 56.53" E	Susceptible
So 524	Witchcliffe, Western Australia	34° 01' 21.14" S	115° 05' 56.53" E	Susceptible
So 527	Pemberton, Western Australia	34° 26' 39.50" S	115° 54' 03.14" E	Susceptible
So 529	Nanarup, Western Australia	34° 59' 37.41" S	118° 03' 48.00" E	Susceptible
So 530	Porongurup, Western Australia	34° 39' 24.00" S	117° 53' 21.22" E	Susceptible
So 532	Mt Barker, Western Australia	34° 38' 02.53" S	117° 39' 59.02" E	Susceptible
So 534	Arthur River, Western Australia	33° 20' 07.59" S	117° 02' 00.22" E	Susceptible
So 541	Bannister, Western Australia	32° 40' 45.14" S	116° 31' 10.08" E	Susceptible

[a] Response to chlorsulfuron - resistant:- 100% survival and no biomass reduction, susceptible: 0% survival and biomass elimination

4.3.5 Victorian *S. oleraceus* chlorsulfuron treatment

In Victoria, 12 accessions were tested (Table 4.6) shows varying sensitivity to chlorsulfuron with 1 accession being resistant and 11 susceptible.

Table 4.6 The collection locations of *S. oleraceus* accessions from Victoria and the response of their progeny to the ALS-inhibiting herbicide, chlorsulfuron (15 g a. i. ha^{-1}).

Sonchus number	Location	Latitude/Longitude		Response to chlorsulfuron [a]
So 254	Horsham, Victoria	36° 42' 45.67" S	142° 12' 04.41" E	Susceptible
So 255	Edenhope, Victoria	36° 53' 56.86" S	141° 30' 59.96" E	Susceptible
So 256	Edenhope, Victoria	36° 55' 38.37" S	141° 27' 16.56" E	Susceptible
So 257	Murtoa, Victoria	36° 34' 57.52" S	142° 28' 14.64" E	Susceptible
So 258	Murtoa, Victoria	36° 33' 15.99" S	142° 28' 36.35" E	Susceptible
So 259	Murtoa, Victoria	36° 33' 05.62" S	142° 29' 37.37" E	Susceptible
So 260	Murtoa, Victoria	36° 33' 65.64" S	142° 29' 37.35" E	Susceptible
So 261	Rupanyup, Victoria	36° 37' 31.40" S	142° 42' 00.88" E	Susceptible
So 262	Rupanyup, Victoria	36° 36' 51.16" S	142° 44' 28.25" E	Susceptible
So 263	Banyena, Victoria	36° 34' 17.56" S	142° 48' 52.82" E	Susceptible
So 264	Sea Lake, Victoria	36° 29' 37.89" S	142° 50' 18.47" E	Susceptible
So 265	Sea Lake, Victoria	35° 28' 45.64" S	142° 48' 22.65" E	Resistant

[a] Response to chlorsulfuron - resistant:- 100% survival and no biomass reduction, susceptible: 0% survival and biomass elimination

4.3.6 Tasmanian *S. oleraceus* chlorsulfuron treatment

A single accession from Tasmanian was tested (Table 4.7) and was susceptible to chlorsulfuron.

Table 4.7 The collection location of *S. oleraceus* accession from Tasmania and the response of its progeny to the ALS-inhibiting herbicide, chlorsulfuron (15 g a. i. ha^{-1}).

Sonchus number	Location	Latitude/Longitude		Response to chlorsulfuron [a]
So 238	Roaches Beach, Tasmania	42° 54' 03.12" S	147° 29' 49.23" E	Susceptible

[a] Response to chlorsulfuron - resistant:- 100% survival and no biomass reduction, susceptible: 0% survival and biomass elimination

The results of testing for resistance are summarised in Table 4.8. In total 274 accessions were tested for resistance to chlorsulfuron, with 44% being resistant to the herbicide. Resistance to chlorsulfuron was discovered in every state of Australia except Tasmania. One accession came from Tasmania and was used for DNA fingerprinting rather than an indication of the level of resitance in the state. The frequency of accessions with resistance to chlorsulfuron varied across states, being most prevalent in Queensland and New South Wales. Relatively high levels of resistance to chlorsulfuron were found in South Australia. The germplasm collection and the DNA extracted from it formed the basis for population relationship studies conducted and discussed in further chapters of this work.

Table 4.8 Susceptibility and resistance of 274 *S. oleraceus* plants treated with the ALS-inhibiting herbicide, chlorsulfuron.

Australian states	Total	Susceptible	Resistant	% Resistant
New South Wales	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%

It should be noted that one accession from Tasmania is not sufficient to provide meaningful data on the resistance level in Tasmania however it was sufficient to provide DNA for genetic comparison purposes.

4.3.7 *S. oleraceus* imazethapyr treatment

Cross resistance between sulfonylurea and imidazolinone herbicides can occur (Tranel and Wright 2002). To identify whether cross-resistance to imidazolinone herbicides was common in *S. oleraceus*, a sample of 15 chlorsulfuron resistant accessions from a single field at Roseworthy in South Australia were treated with 75 g ha^{-1} imazethapyr. All 15 chlorsulfuron resistant plants from a field at Roseworthy, South Australia were found to be susceptible to imidazolinone (Table 4.9).

Table 4.9 Geographic location of 35 plants of *S. oleraceus* collected from South Australia, found to be resistant to chlorsulfuron (15 g a. i. ha^{-1}) and their response to imazethapyr (75 g a. i. ha^{-1}).

Sonchus number	Location	Latitude/Longitude		Response to imazethapyr [a]
So 288	[g]Roseworthy, South Australia	34° 33' 02.50" S	138° 41' 32.50" E	Susceptible
So 289	[g]Roseworthy, South Australia	34° 33' 00.00" S	138° 41' 32.50" E	Susceptible
So 291	[g]Roseworthy, South Australia	34° 33' 00.00" S	138° 41' 27.50" E	Susceptible
So 295	[g]Roseworthy, South Australia	34° 33' 55.00" S	138° 41' 32.50" E	Susceptible
So 302	[g]Roseworthy, South Australia	34° 33' 52.50" S	138° 41' 30.00" E	Susceptible
So 303	[g]Roseworthy, South Australia	34° 32' 52.50" S	138° 41' 32.50" E	Susceptible
So 304	[g]Roseworthy, South Australia	34° 33' 50.00" S	138° 41' 32.50" E	Susceptible
So 308	[g]Roseworthy, South Australia	34° 32' 50.00" S	138° 41' 22.50" E	Susceptible
So 309	[g]Roseworthy, South Australia	34° 32' 50.00" S	138° 41' 20.00" E	Susceptible
So 310	[g]Roseworthy, South Australia	34° 32' 47.50" S	138° 41' 17.50" E	Susceptible
So 311	[g]Roseworthy, South Australia	34° 32' 47.50" S	138° 41' 20.00" E	Susceptible
So 313	[g]Roseworthy, South Australia	34° 32' 47.50" S	138° 41' 25.00" E	Susceptible
So 314	[g]Roseworthy, South Australia	34° 32' 47.50" S	138° 41' 27.50" E	Susceptible
So 319	[g]Roseworthy, South Australia	34° 32' 45.00" S	138° 41' 22.50" E	Susceptible
So 320	[g]Roseworthy, South Australia	34° 32' 45.00" S	138° 41' 20.00" E	Susceptible

[a] Response to imazethapyr - resistant:- 100% survival and no biomass reduction, susceptible: 0% survival and biomass elimination.
[g] Roseworthy, South Australia. Plants from one field (S4).

Often weeds resistant to one sulfonylurea herbicide have cross resistance to other different sulfonylurea herbicides (Powles and Holtum 1994). They may also have resistance to imidazolinone herbicides, depending on the mutation present (Tranel and Wright 2002). In this study all the chlorsulfuron resistant *S. oleraceus* biotypes were found to be susceptible to imazethapyr and this is consistant with the findings of Tranel and Wright (2002) that point mutations in the ALS gene at proline 197 are claimed to result in high levels of sulfonylurea resistance, but little to no imidazolinone resistance.

4.3.8 *S. oleraceus* dose rate experiment

S. oleraceus was found to be very sensitive to chlorsulfuron with adequate control at 1.875 g ha^{-1} well below the recommended rate of 15 g ha^{-1} (Plate 4.1). This high level of sensitivity has been also found in related *Lactuca serriola* (Preston *et al*., 2006).

Plate 4.1 *S. oleraceus* 30 days after treatment with 1.875 g a. i. ha^{-1} chlorsulfuron. Resistant plants on the left and susceptible plants on the right.

Different dose rates produced different responses between *S. oleraceus* populations when treated with chlorsulfuron (Figure 4.1). Susceptible biotypes (103A, 103B, 234 and 56) were completely controlled when treated with and above the normal recommended rate of chlorsulfuron (15 g ha^{-1}) or higher rates. All susceptible biotypes were also controlled at rates of chlorsulfuron well below the recommended rate. This shows that *S. oleraceus* is normally very sensitive to chlorsulfuron. Adequate control was not achieved with biotypes 101, 102, 220, 57 and 125 with the recommended rate of chlorsulfuron. These resistant biotypes survived at rates of up to 120 g ha^{-1} chlorsulfuron (8 times the recommended rate). This finding highlights the fact that increasing herbicide rates will have no benefit in increasing weed kill once resistance has evolved or is present in a field. Seedlings of germplasm 220 were from a single parent and showed consistent high levels of resistance, greater than 80% of the control through to 120 g ha^{-1} chlorsulfuron. Seedlings from germplasm 101, 102, 57 and 125 were from early collections where seed of a number of plants were collected at a location and represent population responses. Because these latter collections were mixed populations, the diminishing dry weights of these latter seedlings when treated with high rates of chlorsulfuron compared with population 220 (Figure 4.1) could be due to a mix of resistant and susceptible plants in the populations tested. However, survival data do not support this hypothesis, because no plants in these populations were killed at the recommended rate of chlorsulfuron (data not shown). Therefore, it is more likely the differences could be explained by different point mutations within ALS in the different populations.

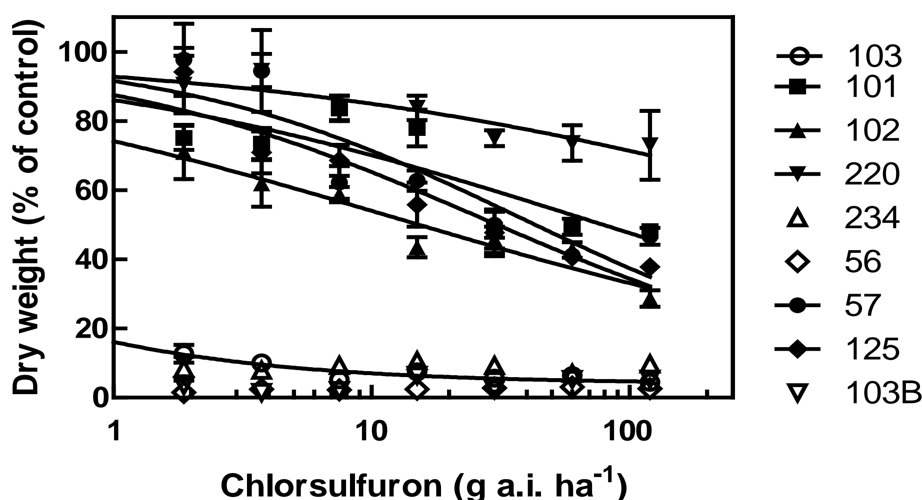

Figure 4.1 Response of *S. oleraceus* treated with chlorsulfuron at different rates. Measurements were made 30 days after treatment. Curves are 103: $Y=4+96/(1+e^{((-1.297-\log dose)*-1.490)})$, 101: $Y=4+96/(1+e^{((-1.811-\log dose)*-0.9781)})$, 102: $Y=4+96/(1+e^{((-1.092-\log dose)*-0.9168)})$, 220: $Y=4+96/(1+e^{((-3.030-\log dose)*-0.83)})$, 234: $Y=4+96/(1+e^{((-53.12-\log dose)*-0.05626)})$, 56 (Function unable to be fitted to these data), 57: $Y=4+96/(1+e^{((-1.577-\log dose)*-1.484)})$ and 125: $Y=4+96/(1+e^{((-1.424-\log dose)*-1.335)})$.

4.4 CONCLUSIONS

ALS-inhibiting herbicide resistance to chlorsulfuron in *S. oleraceus* is now widespread in Australia. From the areas tested there is a much greater frequency of resistance in populations of *S. oleraceus* from southern Queensland and northern New South Wales than in South Australia. *S. oleraceus* plants resistant to the ALS-inhibiting herbicide, chlorsulfuron from a single field in South Australia were all found to be susceptible to the ALS-inhibiting herbicide, imazethapyr. Susceptible *S. oleraceus* populations from a single plant can be very sensitive to the herbicide chlorsulfuron, being controlled by rates as low as 10% of the recommended field rate of this herbicide. Typically, resistant plants can withstand chlorsulfuron treatment of 8 times the recommended rate. Therefore, increasing herbicide rate will not control resistant plants so land managers will need to use alternate strategies to control resistant *S. oleraceus*.

CHAPTER 5

5 GENE MOVEMENT

5.1 INTRODUCTION

Herbicide resistance gene flow may occur through pollen or by seed. Levels of gene flow are known to vary. For example, herbicide tolerant canola has been shown to pollinate susceptible individuals at 2.6 km from the source (Reiger *et al*., 2002). Levels of resistance gene flow of greater than 80% of both glyphosate and glufosinate in resistant roadside canola populations which had escaped from canola field crops have been reported (Knispel *et al*. 2008). In *Medicago sativa* L. (alfalfa or lucerne), which is predominately cross-pollinated, Fitzpatrick (2003) reported gene flow of 1.39% at 500 feet plant separation and a reduction to 0.08% at greater than 1500 feet of separation. Messeguer *et al*., (2001) reported that gene flow due to pollen movement in *Oryza sativa* L. (rice), which is self-pollinated, to be less than 0.1% in plants 5 meters apart however this increased to 0.53% in plants in line with the direction of wind movement. Since *S. oleraceus* is self pollinated it is expected that little if any gene flow will occur by cross pollination. Gene flow introduces new alleles into a population and in this study the interest is the movement of the resistant allele into a plant that is susceptible to ALS-inhibiting herbicides.

Seed set in self-pollinated plants is known to be enhanced by insect visits, as reported by Lu *et al*., (2008) to occur in *Eupatorium adenophorum* (crofton weed). Bees were observed visiting the flowers of *S. oleraceus* in the current study (Plate 5.1); however, it is not known whether any cross pollinated seed was produced by the bee visits. Boda Slotta (2006) noted that suppressing flowering and seed set could achieve the greatest effect in prevention of continued spread and the formation of novel genotypes through pollen and seed dispersal.

Plate 5.1 *S. oleraceus* being visited by a bee.

Davies (2007) outlines the potential fates of a seed via 16 pathways. These pathways and fates included movement to the immediate vicinity of the parent plant, special dispersion, multiple vector dispersal, death and establishment; and suggested wind breaks to reduce wind dispersal. Gene flow in weeds can be due to the movement of farm equipment, contamination of seeds or transport by wildlife or animals, insects, wind or ballistic spread (Ashigh and Tardiff 2007; Davies 2007; St.John-Sweeting and Morris 1991). Shields (2006) using remote piloted model planes with attached seed collection mesh bags collected wind dispersed *Conyza canadensis* (horseweed) seeds at levels of up to 140 meters above the ground and further concluded that seed dispersal can easily exceed 500 km in a single dispersal event. This movement also contributes to the movement of the gene. Management strategy plays an important role in the immigration and extinction level of the genes contained within plant seeds (Liebman 2007).

In this experiment two plants, one resistant and another susceptible to ALS-inhibiting herbicides, were placed side by side in the field and allowed to flower and produce seed

heads. By testing the seed from the susceptible plants for resistance to ALS-inhibiting herbicide the frequency of gene flow can be determined. If gene flow does occur, self pollination of the resistant progeny can be used to see if segregation has occurred and if so determine level of cross pollination. The aim of this experiment was to ascertain whether any gene flow occurred through pollen movement.

5.2 MATERIALS AND METHODS

5.2.1 Experimental site of field experiment

The experiment was conducted in a field at Urrbrae, South Australia, (34° 58' 14" S 138° 38' 23" E), in late spring and early summer 2006 during a period were the plant naturally flowers and natural pollination is maximised.

5.2.2 Germination, seedling and plant treatment

Plants selected for inclusion in the gene flow experiment are listed in Table 5.1. Seed from these accessions was germinated as described in section 3.3.1. Black pots were used for susceptible plants and pink pots for resistant plants to reduce the risk of inadvertently harvesting a resistant head by mistake when harvesting susceptible heads. Seedlings from So115 (Table 5.1) were transplanted to 10 black pots (80 X 80 mm diameter and 180 mm tall) labelled S01 to S10, as described in section 3.3.2. Similarly resistant seedlings from field pair numbers R01 to R10 as listed in Table 5.1 were transplanted to 10 pink pots. All pots were kept in the glasshouse and watered to field capacity for 9 days prior to movement to a bird proof enclosure outside for a further 26 days to acclimatise and reach the point of flowering.

After outside acclimatisation, plants were then placed in the field as shown in Plate 5.2 with a resistant and susceptible plant placed 100 mm apart in a container to hold 50 mm of water for plants to absorb by capillary rise. Each of the ten plant pairs (Table 5.1) were set up as field

stations located 10 to 20 m apart in the field. The plants were placed 100 mm apart to maximise the potential for cross pollination and 10 to 20 m apart to minimise the potential. The flow chart (Figure 5.1) shows the proposed general methodology of the gene flow experiment.

Plate 5.2 Resistant plant (in pink pot) and susceptible plant (in black pot) placed side by side in the field.

Table 5.1. Geographic origin of resistant *S. oleraceus* plants selected for inclusion in the gene flow experiment paired with susceptible plants So1 to So10 which were progeny from *Sonchus oleraceus* accession (So115) from the Adelaide parklands.

Field station pair [a]	*Sonchus* resistant parent identification	Resistant parent collection location
S01 - R01	So 57	Goondiwindi, Queensland
S02 - R02	So 101	Wasleys, South Australia
S03 - R03	So 102	Roseworthy, South Australia
S04 - R04	So 106	Virginia, South Australia
S05 - R05	So 108	Wasleys, South Australia
S06 - R06	So 125	Dalby, Queensland
S07 - R07	So 145	Warra, Queensland
S08 - R08	So 149	Warra, Queensland
S09 - R09	So 154	Meandarra, Queensland
S10 - R10	So 155	Dalby, Queensland

[a] Field pairs consist of two pots with a susceptible plant in one (black) and resistant plant in the other (pink).

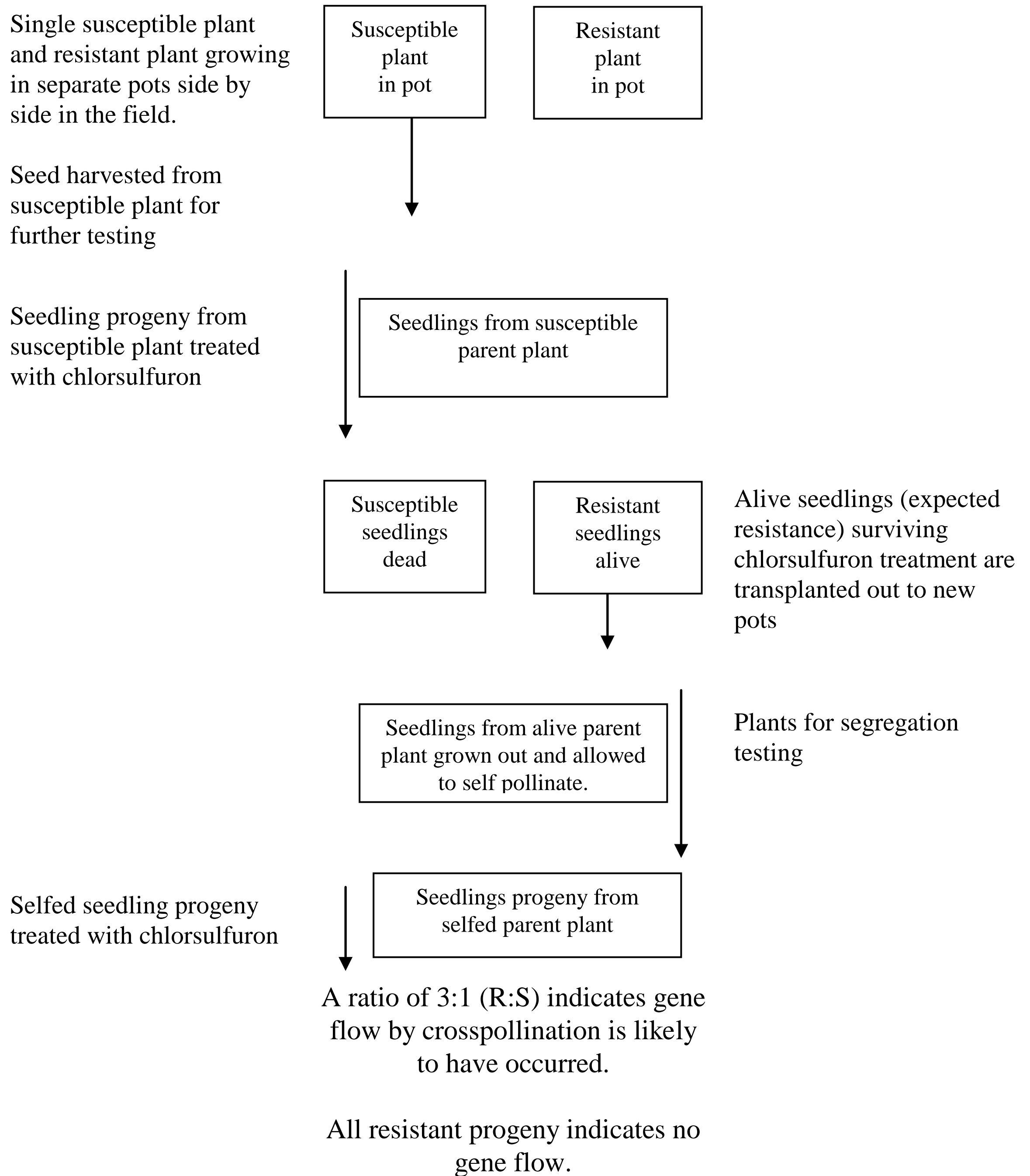

Figure 5.1 Flow chart showing the cultivation and treatment of *S. oleraceus* plants and seedlings within the gene flow experiment.

5.2.3 Measurements

Over a six week period (February and March 2007) flowering heads were tagged daily and the seed heads from both plants were harvested about 10 days after flowering. Seed heads were air dried and stored. All seed from susceptible heads were then germinated as identified lots into Mk12 pots containing coco peat as described in section 3.3.2. Seed from resistant plants was sown into the centre row of the Mk12 pots as a control (Plate 5.3) then treated with chlorsulfuron as section 3.3.5 and measurements made as section 3.3.6.

Plate 5.3 Resistant plants in the centre four pots and susceptible plants sown in the outside four pots to the right and left of the centre column.

5.2.4 Segregation test

Any resistant plants identified in the chlorsulfuron treated progeny from susceptible parents were transplanted to fresh pots and grown to produce self seed. Flowering seed heads were bagged (Plate 5.4) to reduce the chance of insect cross pollination and the subsequent seed produced was harvested, dried and sown in pots as section 3.3.2., for treatment with chlorsulfuron and determination of level of resistance or susceptibility. Eleven progeny were

allowed to self with heads bagged individually, and the progeny of these selfed plants were grown and treated with chlorsulfuron to determine the proportion of resistant and susceptible progeny. A χ^2 test was used to determine whether the segregation of the progeny fitted a 3:1 ratio.

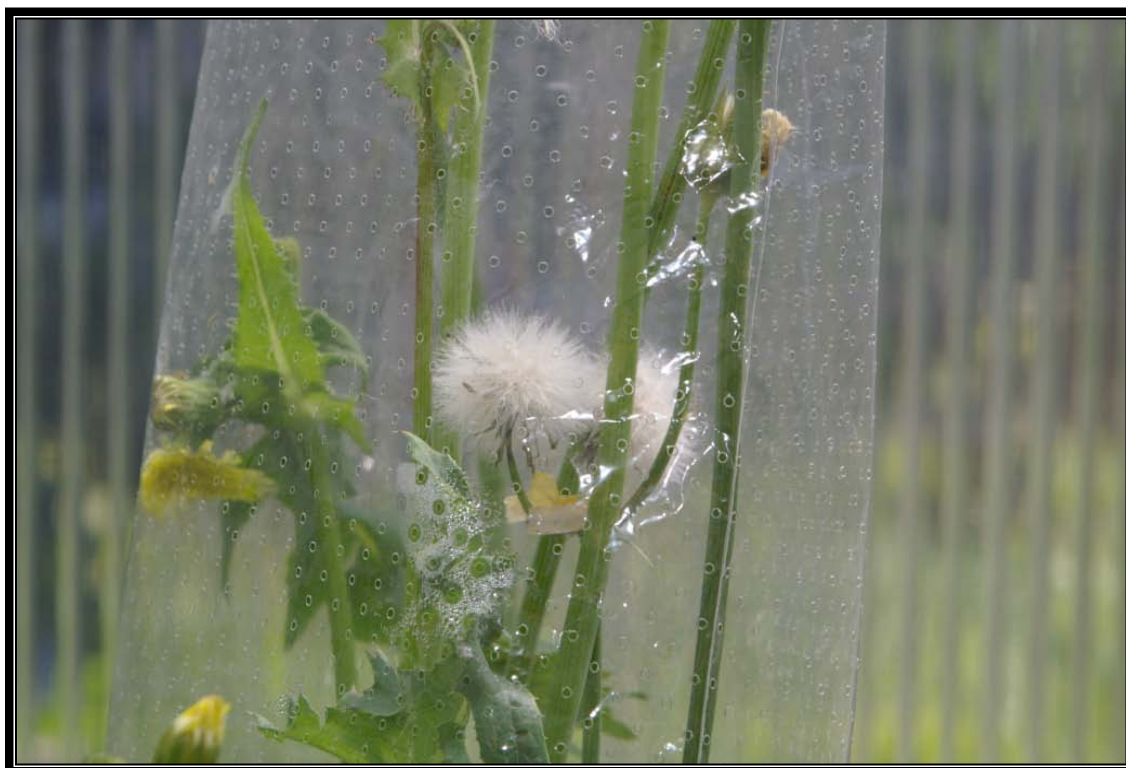

Plate 5.4 A bagged resistant plant which is the progeny from a susceptible plant. The plant is bagged to exclude large insects and reduce the already low chance of cross pollination.

5.3 RESULTS AND DISCUSSION

5.3.1 Field experiment

The results of chlorsulfuron treated seedlings from the seed heads collected from the 10 field station pairs listed in Table 5.1 are displayed in Appendix 2 (S1&R1 to S10&R10). One example from Appendix 2, S1 is displayed in Table 5.2 and shows the 11 head harvested numbers (in the second column) over the flowering period (February and March 2007). Column three shows the number of heads harvested and column five the number of emerged seedlings treated with chlorsulfuron. The last two columns show the results of the

chlorsulfuron treatment with a total of 329 dead and zero living seedlings. The totals from S01 in Table 5.2 appear in the first row of Table 5.3. Table 5.3 displays the results from all 10 stations, with the first line being the totals from the first field pair from Table 5.2. Table 5.3 shows no cross pollination has occurred from the proximate resistant plant So57. Seed from selected resistant plants (R1 to R10, in Appendix 2) were used to confirm parental resistance, with all being confirmed resistant. Seeds collected from the heads of susceptible parent plants of each of the 10 field stations are displayed in Table 5.3 with the level of resistance found in the progeny from the maternal susceptible parent. The 14,144 seeds collected from susceptible parent plants produced 3795 seedlings and of these 10.34% were found to be resistant. This figure is higher than expected, but is biased by results from one plant (S03) as displayed in Table 5.3. By excluding this outlier the cross pollination rate is less than 4%.

Table 5.2 Number of capitulum seed heads harvested from an individual plant of susceptible *S. oleraceus* line So115 grown within 100mm of an individual resistant *S. oleraceus* line So057 over a six week period. The table also displays the number of seed progeny from So115 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

					Result 30 days after treatment	
Susceptible *S. oleraceus* line and head harvest # [a]		# of capitulum heads harvested	Seed # sown	Seedling # treated [b]	# Dead **Susceptible**	# Alive **Resistant**
S01So115	H033	1	80	38	38	0
S01So115	H037	2	100	76	76	0
S01So115	H079	1	0	0	0	0
S01So115	H091	2	30	24	24	0
S01So115	H136	2	140	102	102	0
S01So115	H168	3	160	31	31	0
S01So115	H189	4	100	6	6	0
S01So115	H207	6	200	6	6	0
S01So115	H222	7	100	17	17	0
S01So115	H235	4	100	28	28	0
S01So115	H251	3	30	1	1	0
Totals		35	1040	329	329	0

[a] Capitulum heads harvested as mature from mid February 2007 to late March 2007.
[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

Table 5.3 Number of capitulum seed heads harvested from an individual plant of susceptible *S. oleraceus* line So115 grown within 100mm of an individual resistant *S. oleraceus* line So057 over a six week period. The table also displays the number of seed progeny from So115 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

			Result 30 days after treatment		
S. oleraceus susceptible and resistant pair [a]	Seed # sown	Seedling # treated [b]	# Dead **Susceptible**	# Alive **Resistant**	% **Resistant**
S01So115 & R01So057	1040	329	329	0	0
S02So115 & R02So101	1678	407	383	24	5.90
S03So115 & R03So102	570	191	46	145	75.92
S04So115 & R04So106	700	77	77	0	0
S05So115 & R05So108	2120	640	629	11	1.72
S06So115 & R06So125	2485	530	465	65	12.26
S07So115 & R07So145	2240	657	638	19	2.89
S08So115 & R08So149	1440	91	91	0	0
S09So115 & R09So154	900	382	374	8	2.09
S10So115 & R10So155	995	491	478	13	2.65
sum	14168	3795	3510	285	103.43
Mean	1417	380	351	29	10.34

[a] Pair of plants (susceptible and resistant) flowering in the field 100 mm apart.

[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

5.3.2 Segregation test

Table 5.4 displays the outcome of the selfed segregation test. Segregation of 3:1 (R:S) was expected for a single dominant gene. The progeny of 7 of the selfed plants were composed entirely of resistant individuals (8, 18, 8, 32, 12, 19 and 21) suggesting these were not from the susceptible parent but possibly harvested by error from the resistant plant. Self 1 is most likely to have been progeny of a susceptible plant surviving spraying with herbicide. For the other three selfs, the segregation of the progeny was not significantly different to a 3:1 ratio (Table 5.4)

Table 5.4 The table displays the number of seeds sown from each selfed parent, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15 g a. i. ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

	Selfed parent		Result 30 days after treatment		χ^2	P
Self number	Parent progeny name	Seedlings treated	# Dead **Susceptible**	# Alive **Resistant**		
Self 1	S02So115H015A	45	45	0		
Self 2	S02So115H032A	8	0	8		
Self 3	S02So115H032B	18	0	18		
Self 4	S05So115H150	16	4	12	0	1
Self 5	S05So115H185	8	0	8		
Self 6	S06So115H184A	32	0	32		
Self 7	S06So115H184B	38	5	33	2.84	0.09
Self 8	S07So115H047	22	7	15	0.55	0.46
Self 9	S07So115H111	12	0	12		
Self 10	S07So115H131	19	0	19		
Self 11	S07So115H217	21	0	21		

The movement of weed germplasm by seed is widely known, (Shields *et al.,* 2006, Lu 2005, Sheldon and Burrows 1973 and Davies and Sheley 2007) and gene flow in numerous weeds through pollen is also known (Maxwell and Mortimer 1994). Specific knowledge as the gene movement in *S. oleraceus* up to now has not been clear.

5.4 CONCLUSION

In this study, field pollination in *S. oleraceus* is shown to have resistance cross pollination movement of less than 10% and is more likely to be less than 4% and gene flow of ALS-inhibiting herbicide resistance in *S. oleraceus* is likely to be predominately by seed movement. This supports the premise that gene flow in *S. oleraceus* is predominately by wind-bourne seed movement. However, this study shows that field pollination does occur and even a small proportion of pollen movement from resistant to susceptible plants is notable as it has the potential to increase the level of resistant seed in the seed bank.

CHAPTER 6

6 GENETIC DIVERSITY IN INDIVIDUAL FIELDS

6.1 INTRODUCTION

Resistance to ALS-inhibiting herbicides is complex and evolves as a result of selection for individuals within plant species that can survive the normal rate of herbicide application. Resistance can be attributed to an enhanced ability of plants to metabolize the herbicide (Christopher *et al*., 1992), but most commonly involves an altered form of the target ALS enzyme (Saari *et al*., 1990, 1992 and 1994). Resistant alleles occur at low frequencies within natural populations (Preston and Powles 2002), but the alleles increase in frequency with frequent use of herbicides with 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. *S. oleraceus* is self pollinated, so any gene flow would most likely occur through seed movement. Rapid dispersal of wind blown seed over large distances has been reported in *Lactuca serriola* L. (Lu 2005). *S. oleraceus* has similar wind borne seed and therefore could also be transported over large distances.

Managing gene flow under such conditions can be difficult. For example, Walker *et al.* (2005b) advocate spraying small seedlings and controlling late flushes of *S. oleraceus* in winter crops with effective selective herbicide groups instead of waiting for the first fallow spray after harvest. These strategies will kill plants prior to flowering, reducing the possibility of resistance allele movement. This may not be the only successful method of controlling resistance allele movement and understanding the major mechanisms of gene flow will assist in selecting and manipulating management options to reduce the rate of resistance increase.

In October 2005 whilst collecting seed heads from individual *S. oleraceus* plants from fields in Queensland, a field of barley was found with a high infestation of mature *S. oleraceus*. From this 25 ha field, seed heads from 23 individual plants were sampled to determine whether there was any variation in the frequency of resistance. After treating emerged seedlings from these plants with chlorsulfuron it was found that 40% of the sampled plants in the field were resistant to the herbicide chlorsulfuron and 60% were susceptible.

Following the finding of mixed susceptible and resistant plants in an individual Queensland field, a second field in South Australia containing *S. oleraceus* that could be readily sampled and monitored was identified. In November 2006 a field of faba beans (*Vicia faba*) containing *S. oleraceus*, at approximately 1 plant per m^2, was found 60 km north of Adelaide at Roseworthy. This field was used to investigate the frequency of resistance, and the genetic plus spatial relationship between plants in this population. This field was selected as it was on the Roseworthy Agricultural College farm where detailed field records had been preserved and was readily accessable. DNA was extracted from the 37 individual plants collected in the Roseworthy field and AFLPs were used to determine the relationship of the 37 genotypes. From the extracted DNA of the 15 resitant plants and one susceptible plan sequence analysis was conducted to identify points of mutation.

6.2 MATERIALS AND METHODS

6.2.1 Site of Queensland individual field experiment and seed collection

In 2005, seed from single *S. oleraceus* plants were sampled from a 25 ha field 30 km south east of Chinchilla and 6 km north west of Warra in southern Queensland (26° 53' 54.00" S 150° 52' 14.00" E). This field contained a population of *S. oleraceus* at about 1 plant per m^2 within a crop of barley. One to five seed heads per plant were collected from 23 individual

plants. After collection, seed was dried in a dehydrator at 40°C for 24 hrs. Seed was stored at 3°C until required.

6.2.2 Site of Roseworthy individual field experiment and seed collection

This survey was conducted in a 22 ha field (South 4) located at Roseworthy Agricultural College 60 km north of Adelaide, South Australia (34° 32' 50" S, 138° 41' 25" E). This field was selected due to the high numbers of *S. oleraceus* found in the crop and the availability of an accurate paddock history. The soil in the field is a calcareous loamy mallee with a mean annual rainfall of 440 mm (predominantly of winter incidence). The past use of ALS-inhibiting herbicides within the field is outlined in Table 6.1. Seed was collected from 37 individual *S. oleraceus* plants in November 2006. After collection seed was dried in a dehydrator at 40°C for 24 hrs, and then stored at 3°C until required.

Table 6.1 Paddock history collected from farm records of field South 4 at Roseworthy Agricultural College, South Australia (34° 32' 45" S 138° 41' 20" E).

Year	Crop or Pasture	ALS Inhibiting herbicide	Herbicide application rate
1982	Wheat	Glean	20g/ha
1983	Pasture Medic		
1984	Barley		
1985	Pasture Medic		
1986	Wheat	Glean	20g/ha
1987	Pasture Medic		
1988	Pasture Medic seed		
1989	Oat Medic Vetch		
1990	Pasture Medic		
1991	Pasture Medic seed		
1992	Barley	Ally	7g/ha
1993	Pasture Medic		
1994	Triticale		
1995	Pasture Medic		
1996	Wheat	Ally	7g/ha
1997	Pasture Medic		
1998	Barley	Ally	7g/ha
1999	Oats		
2000	Pasture Medic		
2001	Pasture Medic		
2002	Canola		
2003	Wheat	Ally	5g/ha
2004	Wheat		
2005	Barley		
2006	Faba Beans		

6.2.3 Seedling treatment and measurements

Seeds were germinated, transplanted, cultivated and treated as general methods 3.3.1, 3.3.2 and 3.3.3. Seedlings were treated with 15 g a. i. ha^{-1} chlorsulfuron as method in section 3.3.4. After 30 days, treated seedlings were scored as either dead or alive.

6.2.4 Genomic DNA extraction and AFLP analysis

DNA was extracted (as method in section 3.4.1.2) from the 37 plants collected. AFLP analysis was performed (as method in section 3.4.3) using the extracted DNA.

6.2.5 Primer Design for ALS Gene Sequencing

DNA from a single susceptible (So115) and 2 resistant plants (So302 and So 313) were used to determine which primer pairs would be used to test all plants. Four reactions were performed So115, So302, So313 with a nanopure water control. The reactions were performed with 4 sets of forward and reverse primers as Table 6.2. Each PCR reaction contained approximately 40 ng genomic DNA, 10μM of each forward and reverse primer, 10mM deoxynucleotide triphosphates (dNTPs),), 50mM $MgSO_4$, 1μL (5U/μL) Platinum HiFI Taq and 1X HiFi PCR buffer in a final volume of 25.5μL. PCR reactions were (after preheating to 90°C) subjected to initial 3 minutes denaturation at 94°C; 34 cycles of (30 seconds at 94°C, annealing for 30 seconds at 54°C and extension for 1 minute at 68°C); then a final 7 minutes at 68°C prior to holding at 4°C. After fragment testing and quantification (as section 3.4.3.2) PCR product was sent to the Australian Genome Research Facility (AGRF) in Adelaide for sequence analysis. Primer pair 2-F05: 2R04A gave the most distinct bands and these primers were used throughout.

Table 6.2 Primer sequences used to select a suitable set of forward and reverse primers in the study of the ALS gene mutations in *S. oleraceus*. The shaded text identifies the selected primers used for ALS gene sequencing.

Primer Pair (4 pairs)	Sequence (5'-3')	Targeted Mutation site	Product Size (bp)	Annealing Temperature used (^oC)
1-F05	TCCTCGTCGAAGCCCTCGAGC	P197	907	56
1-R04	TTCTTCCCAATCTCAGCAGAATC			
2-F05	TCCTCGTCGAAGCCCTCGAGC	P197	483	56
2-R04A	CAAGCTGCTGCTGAATATC			
3-F03A	TTCGTCTCCCGATACGCTCCC	P197	953	56
3-R04	TTCTTCCCAATCTCAGCAGAATC			
4-F03A	TTCGTCTCCCGATACGCTCCC	P197	529	56
4-R04A	CAAGCTGCTGCTGAATATC			

6.2.6 DNA Amplification and Sequencing

Source of DNA

Extracted DNA from 21 individual plants was used in this experiment. The DNA of 15 resistant plants and 6 susceptible plants (So293, So294, So297, So317, So321 and So322) came from field South 4 at Roseworthy as listed in Table 6.4. The DNA of a single susceptible plant (So115) from the Adelaide plains was also included as a standard control.

Sequencing

The ALS gene was sequenced to determine the molecular basis for resistance. The two polymerase chain reaction (PCR) forward and reverse primers as highlighted in Table 6.2 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 21 *S. oleraceus* biotypes. A susceptible control was included to confirm the CCC mutation. PCR reactions containing 40ng of genomic DNA were set up as described in section 6.2.6 and sent to the AGRF for sequencing.

6.2.7 Genomic Data analysis

AFLP data was viewed with Genemapper ® (as method in section 3.4.3.7) for the presence and absence of peaks at each loci, and Popgene ® was then used for analysis of genetic variation. Sequence data were analysed using Invitrogen Vector NTI ® bioinformatics software package.

6.3 RESULTS AND DISCUSSION

6.3.1 Queensland individual field

Table 6.3 shows the response of the 23 *S. oleraceus* plants to chlorsulfuron treatment within a single field at Warra, Queensland. The field location of the resistant and susceptible plants shown in Figure 6.1. The finding that 48% of the plants were resistant prompted further investigation about the spatial evolution of resistance in a single field.

Table 6.3 Geographic location of 23 plants of *S. oleraceus* collected on the 18th October 2005 from a field north of Warra, Queensland and their response to chlorsulfuron treatment.

Sonchus number	Location	Latitude/Longitude		Response to chlorsulfuron [a]
So 128	Warra, Queensland	26° 53' 47.59" S	150° 52' 01.49" E	Susceptible
So 129	Warra, Queensland	26° 53' 49.99" S	150° 52' 01.76" E	Susceptible
So 130	Warra, Queensland	26° 53' 52.97" S	150° 52' 01.77" E	Resistant
So 131	Warra, Queensland	26° 53' 53.24" S	150° 52' 04.10" E	Resistant
So 132	Warra, Queensland	26° 53' 53.26" S	150° 52' 06.66" E	Resistant
So 133	Warra, Queensland	26° 53' 53.14" S	150° 52' 09.01" E	Resistant
So 134	Warra, Queensland	26° 53' 56.34" S	150° 52' 09.43" E	Resistant
So 135	Warra, Queensland	26° 53' 59.90" S	150° 52' 11.46" E	Susceptible
So 136	Warra, Queensland	26° 53' 59.56" S	150° 52' 13.53" E	Susceptible
So 137	Warra, Queensland	26° 53' 59.08" S	150° 52' 16.02" E	Susceptible
So 138	Warra, Queensland	26° 54' 01.92" S	150° 52' 16.63" E	Susceptible
So 139	Warra, Queensland	26° 54' 04.49" S	150° 52' 20.89" E	Susceptible
So 140	Warra, Queensland	26° 54' 01.89" S	150° 52' 22.90" E	Susceptible
So 141	Warra, Queensland	26° 53' 59.16" S	150° 52' 24.64" E	Susceptible
So 142	Warra, Queensland	26° 53' 56.43" S	150° 52' 24.19" E	Susceptible
So 143	Warra, Queensland	26° 53' 56.63" S	150° 52' 20.68" E	Resistant
So 144	Warra, Queensland	26° 53' 53.64" S	150° 52' 18.46" E	Susceptible
So 145	Warra, Queensland	26° 53' 54.76" S	150° 52' 14.65" E	Resistant
So 146	Warra, Queensland	26° 53' 51.49" S	150° 52' 13.99" E	Resistant
So 147	Warra, Queensland	26° 53' 47.63" S	150° 52' 12.90" E	Resistant
So 148	Warra, Queensland	26° 53' 47.04" S	150° 52' 07.95" E	Resistant
So 149	Warra, Queensland	26° 53' 45.71" S	150° 52' 04.74" E	Resistant
So 150	Warra, Queensland	26° 53' 45.49" S	150° 52' 00.04" E	Susceptible

[a] Response to chlorsulfuron - resistant:- 100% survival and no biomass reduction, susceptible: 0% survival and biomass elimination.

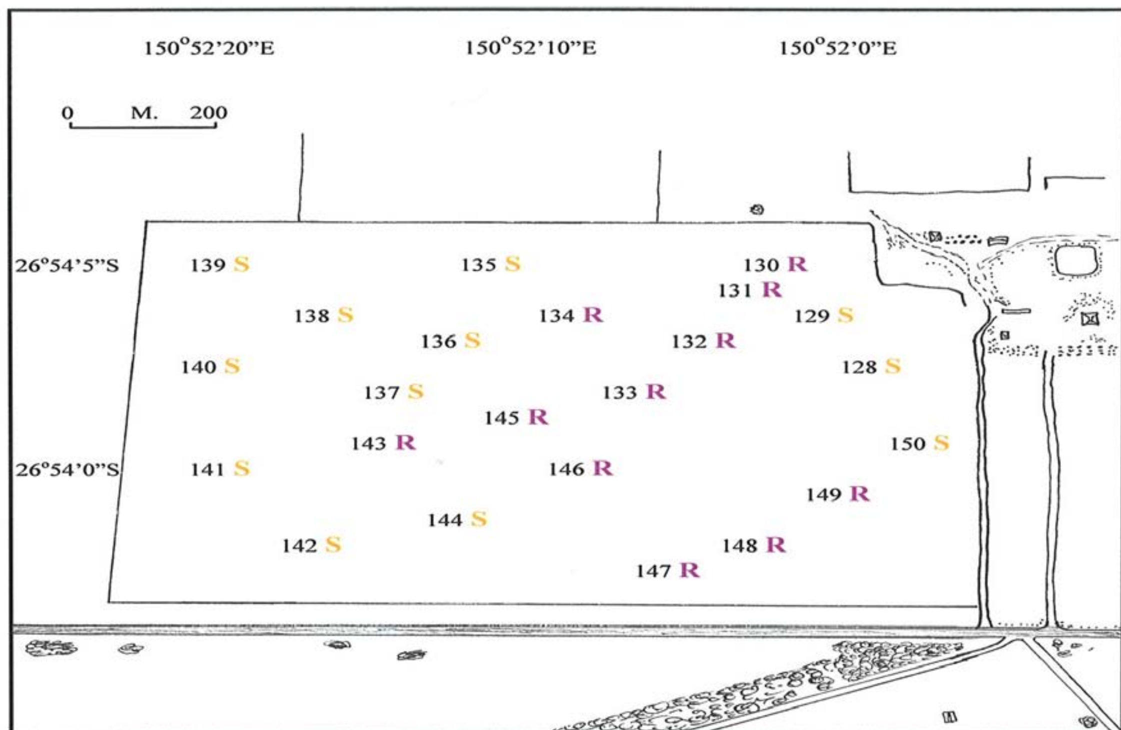

Figure 6.1 Map of a field at Warra, Queensland showing the locations of the 11 resistant plants (pink) and the 12 susceptible plants (yellow).

The finding showed that the hypothesis that *S. oleraceus* plants in an individual field will either be all resistant or all susceptible was unfounded. It is likely that the frequency of resistance will vary in an individual field and this suggests that, because there is a high level of selfing in *S. oleraceus*, populations within fields are founded by more than one parent.

6.3.2 South Australian individual field survey

Resistance to chlorsulfuron was found in the progeny of 15 plants within the S4 field at Roseworthy, South Australia with the other 22 being susceptible (Table 6.4). Resistant and susceptible plant locations within the field are listed in Table 6.4 and displayed in Figure 6.2.

Table 6.4 The geographic location of 37 plants of *S. oleraceus* collected from a single field (South 4) at Roseworthy, South Australia in November 2006 and their response to treatment with the ALS-inhibiting herbicide, chlorsulfuron.

Sonchus number	Location	Latitude/Longitude		Response to chlorsulfuron [a]
So 286	S4, Roseworthy, South Australia	34° 33' 05.00" S	138° 41' 32.00" E	Susceptible
So 287	S4, Roseworthy, South Australia	34° 33' 02.50" S	138° 41' 30.00" E	Susceptible
So 288	S4, Roseworthy, South Australia	34° 33' 02.50" S	138° 41' 32.50" E	Resistant
So 289	S4, Roseworthy, South Australia	34° 33' 00.00" S	138° 41' 32.50" E	Resistant
So 290	S4, Roseworthy, South Australia	34° 33' 00.00" S	138° 41' 30.00" E	Susceptible
So 291	S4, Roseworthy, South Australia	34° 33' 00.00" S	138° 41' 27.50" E	Resistant
So 292	S4, Roseworthy, South Australia	34° 32' 57.50" S	138° 41' 27.50" E	Susceptible
So 293	S4, Roseworthy, South Australia	34° 32' 57.50" S	138° 41' 30.00" E	Susceptible
So 294	S4, Roseworthy, South Australia	34° 32' 57.50" S	138° 41' 32.50" E	Susceptible
So 295	S4, Roseworthy, South Australia	34° 33' 55.00" S	138° 41' 32.50" E	Resistant
So 296	S4, Roseworthy, South Australia	34° 32' 55.00" S	138° 41' 30.00" E	Susceptible
So 297	S4, Roseworthy, South Australia	34° 32' 55.00" S	138° 41' 27.50" E	Susceptible
So 298	S4, Roseworthy, South Australia	34° 32' 55.00" S	138° 41' 25.00" E	Susceptible
So 299	S4, Roseworthy, South Australia	34° 32' 52.50" S	138° 41' 22.50" E	Susceptible
So 300	S4, Roseworthy, South Australia	34° 32' 52.50" S	138° 41' 25.00" E	Susceptible
So 301	S4, Roseworthy, South Australia	34° 32' 52.50" S	138° 41' 27.50" E	Susceptible
So 302	S4, Roseworthy, South Australia	34° 33' 52.50" S	138° 41' 30.00" E	Resistant
So 303	S4, Roseworthy, South Australia	34° 32' 52.50" S	138° 41' 32.50" E	Resistant
So 304	S4, Roseworthy, South Australia	34° 33' 50.00" S	138° 41' 32.50" E	Resistant
So 305	S4, Roseworthy, South Australia	34° 32' 50.00" S	138° 41' 30.00" E	Susceptible
So 306	S4, Roseworthy, South Australia	34° 32' 50.00" S	138° 41' 27.50" E	Susceptible
So 307	S4, Roseworthy, South Australia	34° 32' 50.00" S	138° 41' 25.00" E	Susceptible
So 308	S4, Roseworthy, South Australia	34° 32' 50.00" S	138° 41' 22.50" E	Resistant
So 309	S4, Roseworthy, South Australia	34° 32' 50.00" S	138° 41' 20.00" E	Resistant
So 310	S4, Roseworthy, South Australia	34° 32' 47.50" S	138° 41' 17.50" E	Resistant
So 311	S4, Roseworthy, South Australia	34° 32' 47.50" S	138° 41' 20.00" E	Resistant
So 312	S4, Roseworthy, South Australia	34° 32' 47.50" S	138° 41' 22.50" E	Susceptible
So 313	S4, Roseworthy, South Australia	34° 32' 47.50" S	138° 41' 25.00" E	Resistant
So 314	S4, Roseworthy, South Australia	34° 32' 47.50" S	138° 41' 27.50" E	Resistant
So 315	S4, Roseworthy, South Australia	34° 32' 47.50" S	138° 41' 30.00" E	Susceptible
So 316	S4, Roseworthy, South Australia	34° 32' 45.00" S	138° 41' 30.00" E	Susceptible
So 317	S4, Roseworthy, South Australia	34° 32' 45.00" S	138° 41' 27.50" E	Susceptible
So 318	S4, Roseworthy, South Australia	34° 32' 45.00" S	138° 41' 25.00" E	Susceptible
So 319	S4, Roseworthy, South Australia	34° 32' 45.00" S	138° 41' 22.50" E	Resistant
So 320	S4, Roseworthy, South Australia	34° 32' 45.00" S	138° 41' 20.00" E	Resistant
So 321	S4, Roseworthy, South Australia	34° 32' 45.00" S	138° 41' 17.50" E	Susceptible
So 322	S4, Roseworthy, South Australia	34° 32' 45.00" S	138° 41' 15.00" E	Susceptible

[a] Response to chlorsulfuron - resistant:- 100% survival and no biomass reduction, susceptible: 0% survival and biomass elimination.

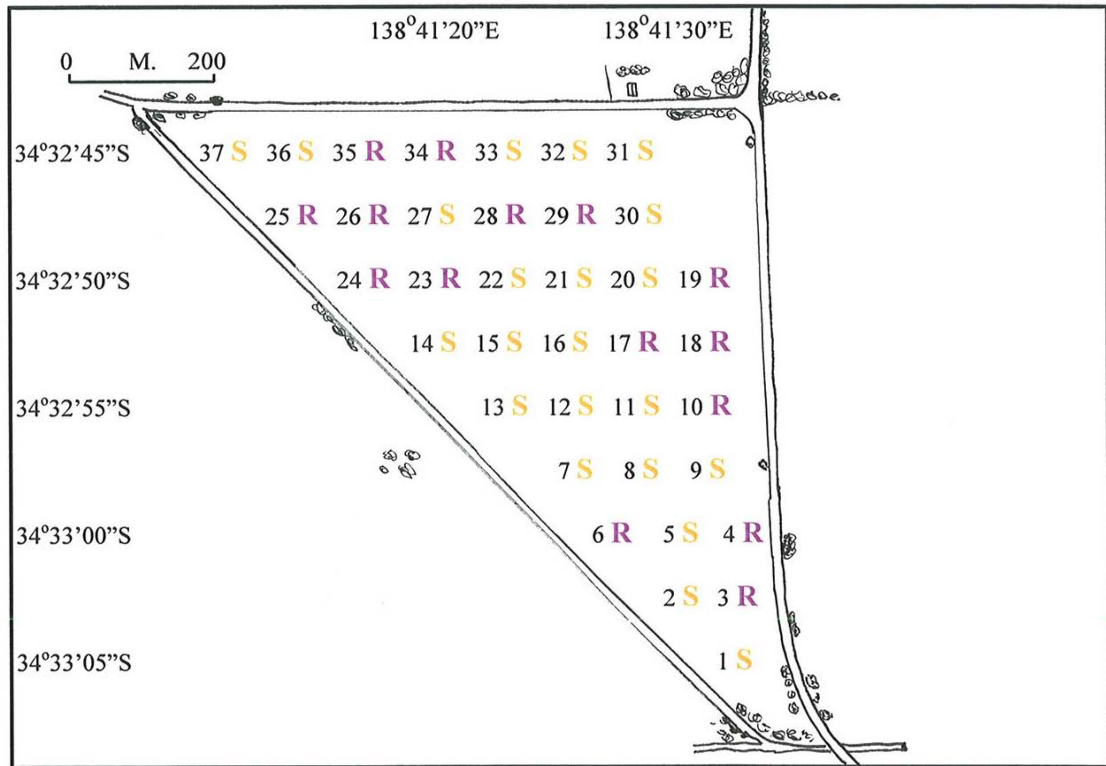

Figure 6.2 Map of field (south 4) at Roseworthy, South Australia showing the locations of the 15 resistant plants (R) and the 22 susceptible plants (S).

Of the plants collected from the single field at Roseworthy, South Australia 59% were found to be susceptible to the ALS-inhibiting herbicide, chlorsulfuron and 41% were found to be resistant. This was slightly lower level of resistance to the findings of the single field at Warra in Queensland (section 6.3.1), where 52% were found to be susceptible and 48% resistant. These findings show that it is important to sample multiple plants from across a field to determine an estimate of the frequency of the resistance allele.

AFLP analysis produced the dendrogram (Figure 6.3), which shows the 15 resistant plants from the field at Roseworthy, South Australia fall into 4 separate genetic groups or clusters. The four groups of resistant genotypes (R1, R2, R3 and R4) determined from the AFLP analysis tended to cluster together within the field (Figure 6.4), but some were distributed more widely. This suggests independent evolution of resistance has occurred in several

individuals from a number of mutation events. This could have occurred in situ, but may also have resulted from wind blown seed successfully colonizing patches in the field. The location of the resistant individuals within the field suggests wind blown seed has probably played a role in the spread of chlorsulfuron resistant *S. oleraceus*, as related biotypes are dispersed within the field.

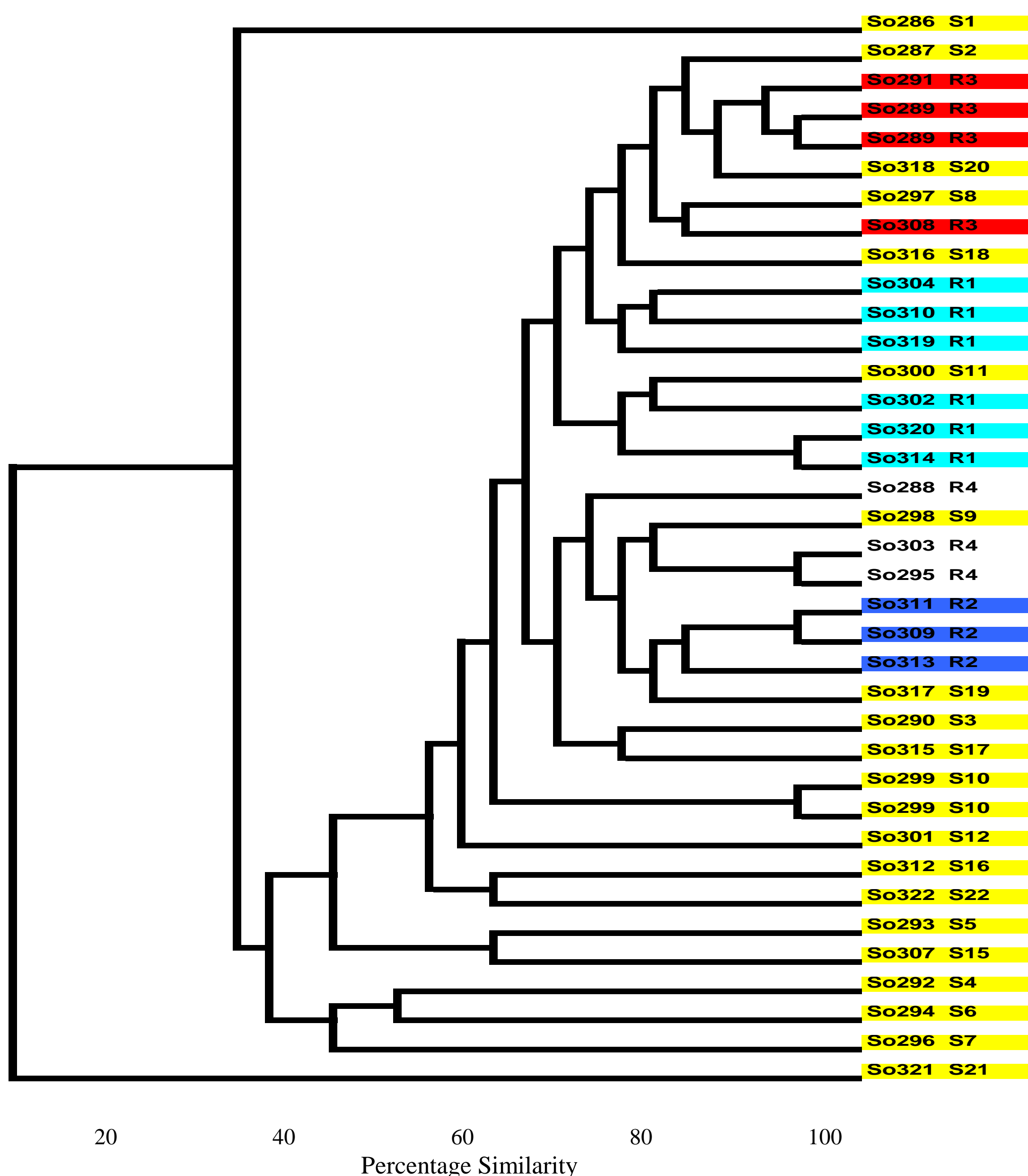

Figure 6.3 Dendrogram showing four separate genetic groups (biotypes) of resistant plants (R1 (aqua), R2 (blue), R3 (red) and R4 (white)) and 22 susceptible plants (yellow).

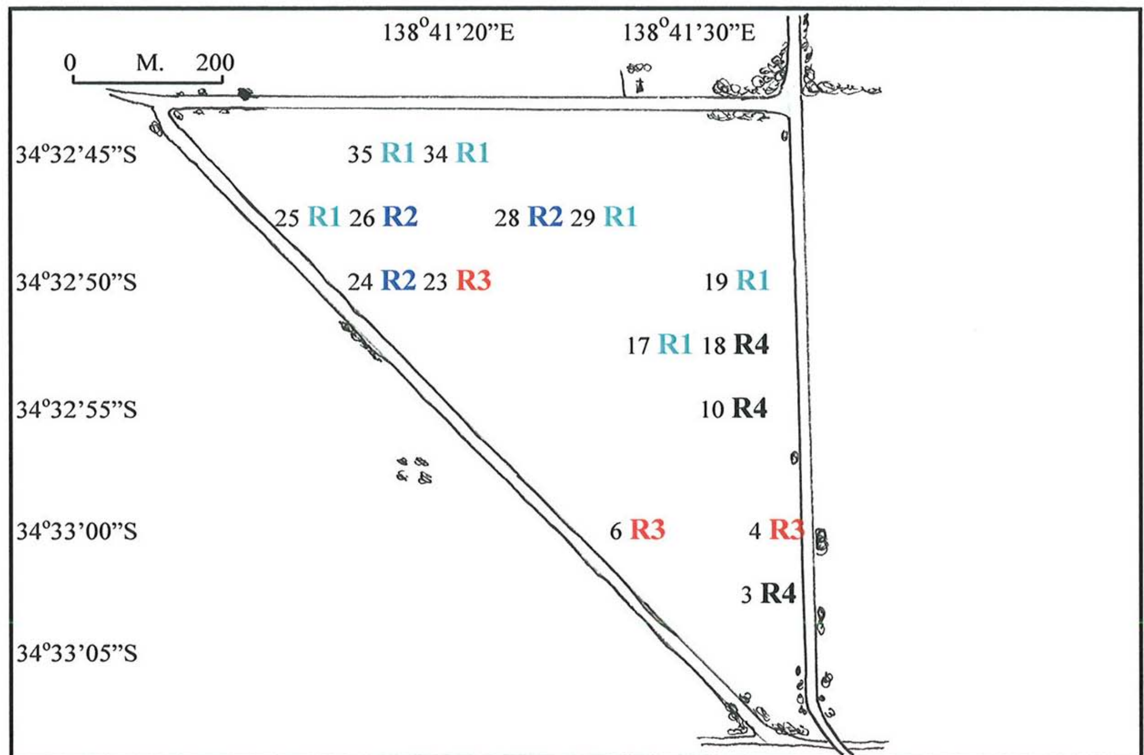

Figure 6.4 Map of the field (south 4) at Roseworthy, South Australia showing the locations of the 15 resistant plants with different genotypes indicated (**R1**, **R2**, **R3** and **R4**).

Given that *S. oleraceus* is primarily a self-pollinated species, the presence of resistant and susceptible plants in a field emphasizes the need for farmers to continue to maintain a zero-tolerance approach to localized infestations and use alternate control methods if a resistant population is detected. This new knowledge provides opportunities for improving current management practices.

As *S. oleraceus* is self pollinated with wind dispersed seed, information from other similar species can be used to increase the understanding of the genetic relationships. Genetic relationships were investigated in a similar wind dispersed self pollinating plant, *L serriola* (Lu *et al*., 2007). Lu *et al*., 2007 found that 25 *L. serriola* plants collected over less than 5 km were identical and collections over a larger area had greater genetic variance. Seed movement as far as 43 km was also reported. This study from a single field shows different genotypes growing together, which may reflect a larger genetic variation in the founder population or a

level of outcrossing. The potential for outcrossing is supported by the finding from section 5.3 which showed outcrossing levels of between 0 to 10% can occur.

To elucidate this sequencing was conducted as described in section 6.3.3 to determine if the mutations occurring within the field were identical, evolved independently from similar genotypes already present or if incoming seed, with different genotypes and mutations, established the resistant patches.

6.3.3 ALS SEQUENCING

The four primer sets tested from Table 6.2 are shown in the Gel Plate (Figure 6.5) with a water control (W). The second set in the top three wells lanes 4, 5 and 6 (Figure 6.5) were selected for use in further sequencing analysis. Low molecular DNA mass ladder bands (M) from top down are 2000bp, 1200bp, 800bp, 400bp, 200bp and 100bp. Prominent distinct bands in the second primer pair are at 483 bp (Reverse 4A [532bp] minus Forward 5 [49bp] = 483bp).

Primer pair 2 (F05 & R04A) as shown (grey background) in Table 6.2 and Lanes 4, 5 and 6 in Gel (Figure 6.5) was selected to sequence 21 individual plants in Section 6.2.6 due to its clear distinct banding pattern.

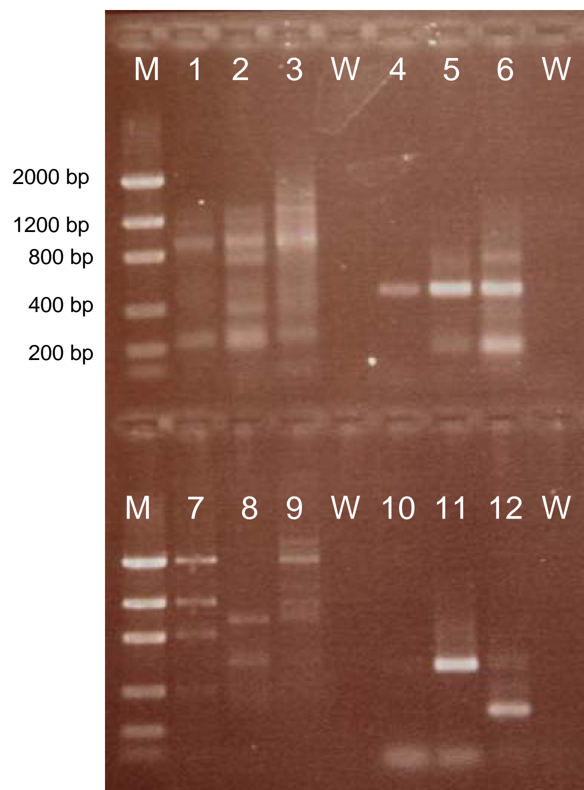

Figure 6.5 Four primer pairs tested with a water control (W) first set in top wells 1, 2 & 3, second set in top wells 4, 5 & 6, third set in bottom wells 7, 8 &9 and fourth set in bottom wells 10, 11 & 12. Susceptible DNA (So115) in lanes 1 and 4 in the top wells and lanes 7 and 10 in the bottom well columns, resistant DNA (So302) in lanes 2, 5, 8 and 11 and resistant DNA (So313) in lanes 3, 6, 9 and 12.

The results of the sequencing show that resistant AFLP clusters R1 (Aqua) and R2 (Blue) (Figure 6.4) had the same mutation (Table 6.5) Proline to Threonine. These clusters are spatially close in the field despite being grouped distantly in the AFLP analysis (Figure 6.3): R3 (Red) and R4 (White/Black) were also close in the field (Figure 6.4) and had one different mutation, proline to leucine (Table 6.5). In five plants no mutation within the part of the ALS gene sequenced was identified. These plants may have had a mutation elsewhere within the ALS gene or may have a different mechanism of resistance, but like R1 (Aqua) and R3 (Blue) appeared as distinct clusters in the AFLP analysis.

Table 6.5 Resistant plants from field South 4 with their response to chlorsulfuron and sequence outcome. Also shown are five susceptible plants from South 4 and one from the Adelaide parklands as a standard control.

S. oleraceus Plant number	South4 Field Cluster from AFLP See Dendrogram Fig 19	Phenotype (resistance profile) Chlorsulfuron (20g ha^{-1})	Proline 197 CCC sequence		
So320	R1 (**Aqua**)	Resistant	**ACC**[a]	Threonine	Resistant
So319	R1	Resistant	**ACC**[a]	Threonine	Resistant
So314	R1	Resistant	CCC	Proline	Susceptible
So310	R1	Resistant	**ACC**[a]	Threonine	Resistant
So304	R1	Resistant	**ACC**[a]	Threonine	Resistant
So302	R1	Resistant	**ACC**[a]	Threonine	Resistant
So309	R2 (**Blue**)	Resistant	**ACC**[a]	Threonine	Resistant
So311	R2	Resistant	**ACC**[a]	Threonine	Resistant
So313	R2	Resistant	**ACC**[a]	Threonine	Resistant
So289	R3 (**Red**)	Resistant	CCC	Proline	Susceptible
So291	R3	Resistant	CCC	Proline	Susceptible
So308	R3	Resistant	CCC	Proline	Susceptible
So288	R4 (White)	Resistant	**CTC**	Leucine	Resistant
So295	R4	Resistant	CCC	Proline	Susceptible
So303	R4	Resistant	CCC	Proline	Susceptible
So115	S (**Yellow**)	Susceptible	CCC	Proline	
So293	S (**Yellow**)	Susceptible	CCC	Proline	
So294	S (**Yellow**)	Susceptible	CCC	Proline	
So297	S (**Yellow**)	Susceptible	CCC	Proline	
So317	S (**Yellow**)	Susceptible	CCC	Proline	
So322	S (**Yellow**)	Susceptible	CCC	Proline	

[a] Mutant resistant alleles are in bold.

6.4 CONCLUSION

S. oleraceus plants resistant to ALS-inhibiting herbicides in a single field have evolved resitance from more than one mutation or mechanism. Although AFLP analysis was helpful in determining the presence of distinct clusters further sequencing analysis found at least 2 different mutations, plus 1 unidentified or different resistance mechanism conferring resistance in the 4 resistant clusters identified in the AFLP analysis.

CHAPTER 7

7 GENETIC DIVERSITY IN AUSTRALIA

7.1 INTRODUCTION

Rapid and less expensive molecular marker systems linked with easy to use software to analyse molecular data has increased the number of published studies examining genetic structure in weed populations (Ward and Jasieniuk 2009). However, Ward and Jasieniuk (2009) noted that in 39 molecular technique and genetic diversity studies published, researchers paid scant attention to sampling design. As discussed earlier, all seed accession collected in this study were from single individuals. Seed from each individual plant may have been collected from one or a number of seed heads. Since *S. oleraceus* is self pollinated, all progeny from this seed is expected to have the same genetic makeup. This was shown to be true in Chapter 4 in that rarely were plants grown from a single parent not either all resistant or susceptible to chlorsulfuron. This high level of self pollination means that seed movement is the most likely 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). *S. 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. *S. oleraceus* seed has little dormancy and readily germinates (Widderick 2002), so the gene pool changes rapidly within sites, enhancing the evolution of resistant populations when selective pressure is present.

Little is known about wind dispersal of the ALS-inhibiting resistance alleles through seed movement. An understanding of the evolution and dispersal of these alleles is likely to be

helpful in not only in managing resistant *S. oleraceus*, but also in understanding resistance to other herbicides in self pollinated and wind dispersed weeds.

S. 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 gene flow of *S. oleraceus* with a view to develop 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 Australian states and provide an opportunity for improving current management practices.

7.2 MATERIALS AND METHODS

7.2.1 Seed collections and treatment

Seed from individual plant accessions were collected throughout Australia between 1997 and 2008. Plants were collected from 50 m diameter patches within fields or along roadsides. At least 5 plant samples were taken from a sampling patch or location. One to five capitulum seed heads per plant were collected with each location recorded using a Global Positioning System (GPS). Plant seed collection and storage method is detailed in section 3.2. 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^{-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).

7.2.2 DNA extraction and Amplified Fragment Length Polymorphisms

A selection of 34 plants from across the country, including some from within the same field were used for AFLP analysis. 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 M*se*1 and P*st*1 restriction enzymes, consequently M*se*1 and P*st*1 adapters were used in the ligation step. Pre-amplification PCR used P*st*1+A and M*se*1+C with the selective PCR amplification using dimers of P*st*1+A and M*se*1+C sequences (M*se*1+CC and P*st*1+AC: M*se*1+CT and P*st*1+AG). P*st*1+AC and P*st*1+AG dimer primers were fluorescently labeled. 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).

7.2.3 Population analysis

Genomic data was viewed with GeneMapper® software for the presence or absence of peaks which were analysed using PopGene® software to determine genetic relationships. The method is detailed in section 3.4.3.6 under genetic data analysis.

7.3 RESULTS AND DISCUSSION

A key aspect of this study is that each *S. oleraceus* accession is from an individual plant. This is a critical point of note as it allows a clear understanding of expected outcomes from the seed without the complications of mixed sampling techniques as discussed by Ward and Jasieniuk (2009).

A study of 34 susceptible and resistant individuals (Table 7.1) sourced from several populations across Australia were selected for AFLP analysis. Despite some evidence of geographic clustering a dendrogram produced from these individuals (Figure 7.1) using 255 scored loci shows large genetic diversity present in Australia. It also shows the presence of several different resistant genotypes. For example genotypes from South Australia cluster with those from Queensland (Clusters A1 and A2) and also include both resistant and susceptible plants. The geographic distance from the Western Australian cluster B1a1a2 and the South Australian cluster B1a1a1 was 2100 km, however the genetic distance between these two clusters was were small. Geographic distances between South Australian and Queensland, South Australian and New South Wales, South Australian and Victorian and Tasmanian and Queensland clusters were 1400 km, 1200 km, 400 km and 1600 km respectively and there was a close genetic relationship in some populations over these large distances.

Although seed can be moved great distances via wind-dispersal, it is unlikely that resistant genotypes would be moved between Queensland and Victoria 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. Subsequent work, not reported here, looked at whether resistant populations had the same mutations reported by Ashigh and Tardif (2007) and whether there was a geographical correlation in identified mutations in Australian plants. Different mutations, some occurring in the same location, were identified, supporting the supposition that *S. oleraceus* has evolved resistance to ALS-inhibiting herbicides as a result of independent mutation events on multiple occasions in Australia (Section 6.3.3).

Table 7.1 Geographic location of *S. oleraceus* plant accessions collected throughout Australia selected for inclusion in the genetic diversity study and their response to the recommended field rate of chlorsulfuron 15g ai ha^{-1}

Sonchus number	Location	Latitude/Longitude		Response to chlorsulfuron [a.]
So 14	Dalby, Queensland	27° 08' 93.00" S	151° 17' 11.00" E	Resistant
So 17	Gatton, Queensland	27° 33' 34.00" S	152° 16' 46.00" E	Susceptible
So 57	[b.] Goondiwindi, Queensland	28° 32' 49.33" S	150° 18' 26.66" E	Resistant
So 101	Wasleys, South Australia	34° 28' 51.65" S	138° 41' 12.81" E	Resistant
So 103	Nairne, South Australia	35° 02' 42.44" S	138° 54' 43.40" E	Susceptible
So 115	Adelaide, South Australia	34° 56' 35.35" S	138° 34' 53.29" E	Susceptible
So 125	Dalby, Queensland	27° 08' 93.00" S	151° 17' 11.00" E	Resistant
So 142	Warra, Queensland	26° 53' 16.00" S	150° 51' 37.00" E	Susceptible
So 154	Meandarra, Queensland	27° 22' 00.00" S	150° 04' 71.00" E	Resistant
So 159	Billa Billa, Queensland	28° 10' 00.00" S	150° 27' 00.00" E	Resistant
So 172	North Star, New South Wales	28° 52' 76.00" S	150° 23' 96.00" E	Resistant
So 173	Yallaroi, New South Wales	28° 08' 03.00" S	150° 22' 27.00" E	Resistant
So 176	Yetman, New South Wales	28° 55' 50.00" S	150° 46' 12.00" E	Resistant
So 238	Roaches Beach, Tasmania	42° 54' 03.12" S	147° 29' 49.23" E	Susceptible
So 241	Port Lincoln, South Australia	34° 44' 46.03" S	135° 52' 45.74" E	Susceptible
So 254	Horsham, Victoria	36° 42' 45.67" S	142° 12' 04.41" E	Susceptible
So 256	Edenhope, Victoria	36° 55' 38.37" S	141° 27' 16.56" E	Susceptible
So 257	Murtoa, Victoria	36° 34' 57.52" S	142° 28' 14.64" E	Susceptible
So 265	Sea Lake, Victoria	35° 28' 45.64" S	142° 48' 22.65" E	Resistant
So 285	Baratta, South Australia	31° 56' 09.54" S	139° 05' 44.64" E	Susceptible
So 289	Roseworthy, South Australia	34° 33' 00.00" S	138° 41' 32.50" E	Resistant
So 299	Roseworthy, South Australia	34° 32' 52.50" S	138° 41' 22.50" E	Susceptible
So 401	North Star, New South Wales	28° 53' 01.00" S	150° 23' 59.00" E	Resistant
So 402	North Star, New South Wales	28° 53' 02.00" S	150° 23' 58.00" E	Resistant
So 408A	North Star, New South Wales	28° 54' 49.00" S	150° 23' 40.00" E	Resistant
So 408B	North Star, New South Wales	28° 54' 49.00" S	150° 23' 40.00" E	Resistant
So 413	North Star, New South Wales	28° 56' 27.00" S	150° 24' 05.00" E	Susceptible
So 431	Toowoomba, Queensland	27° 35' 18.00" S	151° 56' 54.00" E	Susceptible
So 509	Busselton, Western Australia	33° 39' 24.40" S	115° 24' 42.32" E	Resistant
So 523	Witchcliffe, Western Australia	34° 01' 21.14" S	115° 05' 56.53" E	Susceptible
So 530	Porongurup, Western Australia	34° 39' 24.00" S	117° 53' 21.22" E	Susceptible
So 534	Arthur River, Western Australia	33° 20' 07.59" S	117° 02' 00.22" E	Susceptible
So 555	Adelaide, South Australia	34° 55' 57.52" S	138° 35' 47.11" E	Susceptible
So 562	Roseworthy, South Australia	34° 32' 59.28" S	138° 41' 32.87" E	Susceptible

[a] Response to chlorsulfuron - resistant:- 100% survival and no biomass reduction, susceptible: 0% survival and biomass elimination

[b] Goondiwindi, Queensland. First ALS-inhibiting herbicide resistant plant found in Australia in 1991.

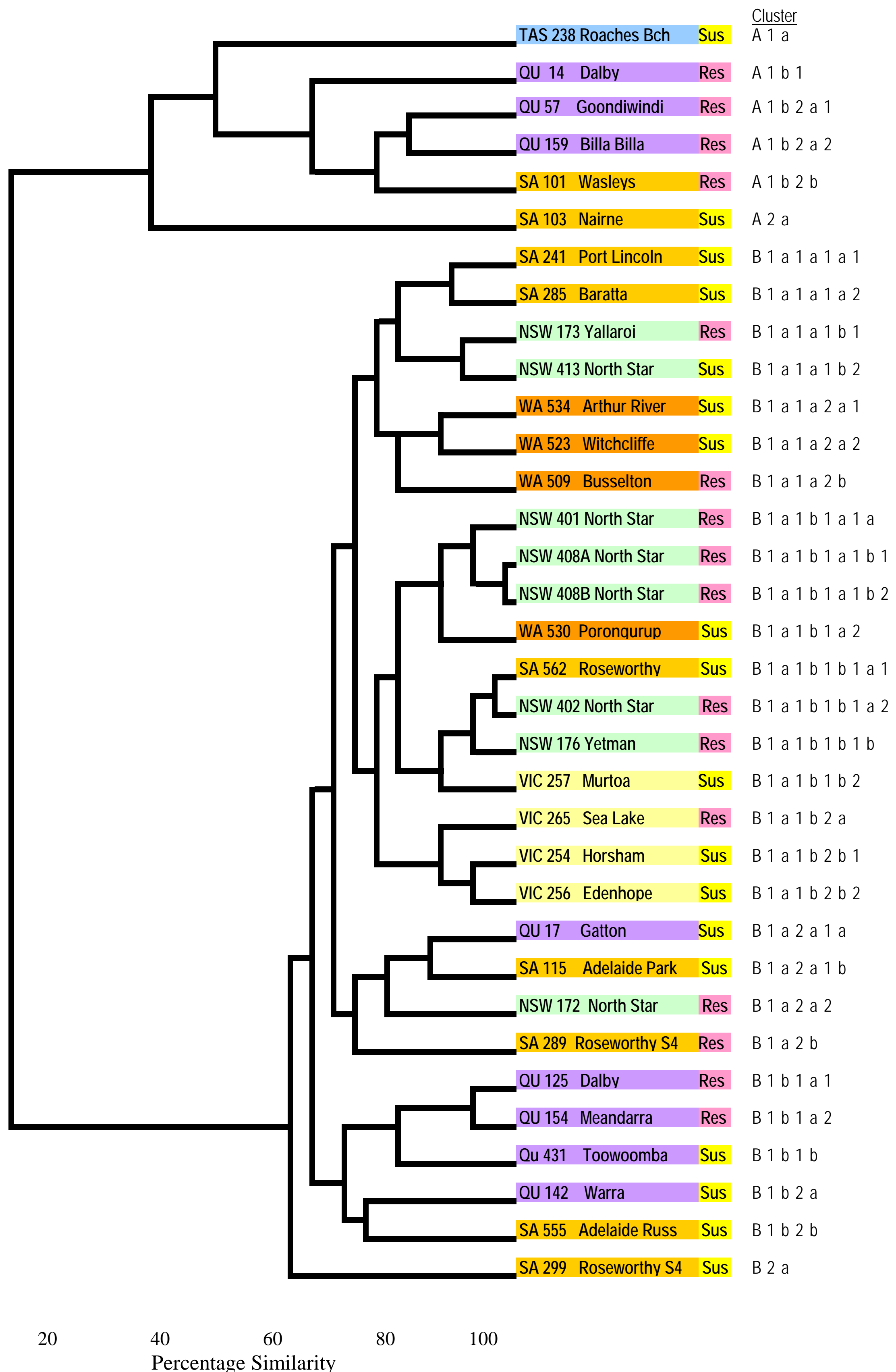

Figure 7.1 Dendrogram of 34 individuals produced from 255 scored loci using Popgene ® software. Resistant (Res) and Susceptible (Sus) plants were from South Australia (SA), Queensland (Qu), Victoria (Vic), New South Wales (NSW) Western Australia (WA) and Tasmania (Tas).

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

7.4 CONCLUSION

There is large genetic diversity in *S. oleraceus* in Australia and seed has been dispersed across large distances. This has facilitated the movement of the resistant gene in addition to numerous independent mutation events. The results presented here show that field pollination does occur and could result in pollen movement from resistant to susceptible plants. While this does have the potential to increase the level of resitant seed in the seed bank the predominant movement of R alleles is through seed dispersal, most likely wind or human mediated.

CHAPTER 8

8 GENERAL DISCUSSION

Worldwide, weeds are evolving resistance to many herbicides, which poses a great challenge to herbicide sustainability in world agriculture (Powles and Yu 2010). The evolution of herbicide resistance demonstrates that over-reliance on any single weed management tool will cause that tool to fail (Powles and Shaner 2001). For example, the widespread adoption of sulfonylurea herbicides across farming areas from the 1980s has resulted in selection within weed populations for resistance to ALS-inhibiting herbicides (Preston *et al*., 2006). Herbicide resistance reduces the economic viability of farming due to the high cost of herbicides, which are ineffective in controlling resistant weeds and the costs of alternate weed control strategies. Once resistance has evolved, the movement of seed from that resistant plant can spread the problem across the landscape. For example, when weather conditions are conducive to the dispersal of *S. oleraceus* seed, the pappus bearing seed is dispersed by wind over both short distances (within metres) and long distances (hundreds of kilometres) Lu *et al*., (2007).

This study has investigated how resistance alleles might move in a plant with wind dispersed seeds. Little was known about how widespread ALS-inhibiting herbicide resistance in *S. oleraceus* is in Australia. In addition, it was not known how frequently resistance to ALS-inhibiting herbicides was evolving, what the likely impact of dispersal of resistance alleles has on the spread of resistance once evolved and how far migration of seed could occur. This work is important as it provides knowledge to make informed decisions as to the best management strategies particularly for wind dispersed weeds.

In this study a dose rate experiment, treating resistant and susceptible biotypes of *S. oleraceus* with different rates of chlorsulfuron, found the susceptible *S. oleraceus* could be controlled with as little as 10% of the recommended field rate of this herbicide. This experiment also

found that resistant *S. oleraceus* plants could withstand 8 times the recommended field rate of chlorsulfuron. This result highlights the high levels of resistance to this herbicide that can occur in *S. oleraceus* and the likely problems controlling this weed.

Seed of *S. oleraceus* was collected from plants across Australia as a basis for this study. Plants grown from this seed were treated with the ALS-inhibiting herbicide, chlorsulfuron at the recommended field rate to ascertain the level of resistance. The Australia wide survey found that 44% of the 274 accessions treated were resistant to chlorsulfuron and resistance was particularly prevalent in Queensland (59%) and New South Wales (78%), states with intensive summer fallow programs (Chapter 4). Similar findings have been found in surveys with other weed species such as *Lactuca serriola* (Mallory-Smith *et al*., 1990a) and numerous other weed species (Heap 2010). This finding is consistent with world wide research findings (Powles and Yu 2010), although the frequency of resistant populations in *S. oleraceus* is particularly high in comparison with other plant species (Powles pers. comm. 2011). The high frequency of resistance found in this study is of concern and will need to be addressed to reverse the trend of increasing resistance and to control the weed. The problem with *S. oleraceus* illustrates the greater problems of resistance with wind blown seed. Wind blown seed is known to disperse widely across the landscape. In contrast, dispersal of resistance pollen is likely to occur over small distances due to pollen viability decaying rapidly and the need for a recipient plant, although it is known that wind borne pollen can travel long distances, with 20 km being recorded (Watrud *et al*., 2004). The collection of seed from individual plant accessions in this study proved to be a very valuable method as more detailed and accurate conclusions were able to be drawn from the results; this was particularly valuable in the gene flow experiment as variations could be precisely explained.

Weed spread can occur by numerous methods, such as wind, in manure, through equipment and contamination in seed (Thill and Mallory-Smith 1997, Llewellyn and Allen 2006, Boyd and White 2009). Gene flow in a plant population is capable of causing evolutionary change through emigration or immigration (Radosevich *et al.*, 1991). Therefore, an increased knowledge of gene flow in a specific plant species is beneficial in formulating strategies to combat the problem of herbicide resistance. Gene flow of ALS-inhibiting herbicide resistance in *S. oleraceus* is clearly by seed movement with field pollination in *S. oleraceus* being shown in this study as less than 10% and is more likely to be less than 4% after removing an outlyer (Chapter 5). These findings are consistent with low levels of cross pollination in most self pollinated plants, rice < 1% (Messeguer *et al.*, 2001), barley grass (Baker *et al.*, 2005) and eastern black nightshade (Ashigh *et al.*, 2008).

Hordeum glaucum (barley grass) is an obligate self pollinator, with different gene profiles indicating independent mutations causing the evolution of resistance (Baker *et al.*, 2005). Similarly, because *S. oleraceus* is self pollinated any gene flow would most likely occur through seed movement. This means it would be possible to track individual plants to determine genetic diversity on a close (field) and large landscape scale (Australia).

Although genomic technologies have a proven record in advancing plant biological knowledge, they are not without their difficulties such as software with numerous shortcomings including lack of user-friendly functionality (Stewart *et al.,* 2009). The use of molecular markers to investigate herbicide resistance in weed populations has the potential to influence weed management strategies by providing a clearer understanding of resistance mechanisms (Boda Slotta 2008). Molecular methods can be used to explain the potential for gene flow in herbicide resistant or genetically modified organisms.

The individual field work in this study utilised genomic molecular technologies and found that a number of different *S. oleraceus* genotype clusters resistant to ALS-inhibiting herbicides and different mutations can exist within a single field (Chapter 6). Mutations within the ALS protein sequence resulting in resistance to herbicides are known at seven different sites in plants (Powles and Yu 2010). One of the most common of these mutations is at Proline 197 and it appears that any amino acid substitutions at this proline residue will result in an active enzyme that is resistant to chlorsulfuron (Preston *et al*., 2006). The threonine to proline 197 substitution found in this study have also been observed in *Lactuca serriola* (Preston *et al*., 2006), *Kochia scoparia* (Guittieri *et al*., 1995, Warwick *et al*., 2008) and *Papaver rhoeas* (Scarabel *et al*., 2004). In a single field in this present study it was found that 2 different mutations occurred plus an unidentified mutation or mechanism.

There is large genetic diversity in *S. oleraceus* in Australia and seed has been dispersed across large distances, which has facilitated the movement of resistance alleles in addition to independent mutation events. Increasing the chlorsulfuron herbicide rate will not control resistant plants and land managers will need to use alternate control strategies for resistant *S. oleraceus*. It is important to know the genetic diversity of *S. oleraceus* in Australia, as this knowledge gives an insight into the plant's ability to cope with environmental variability. Genetic diversity is the primary basis from which *S. oleraceus* can adapt to changes in the environment over time, such as differing herbicide exposures (Tranel and Wright 2002). From the geographic diversity analysis of plants (34) collected from different states in Australia a large genetic diversity of *S. oleraceus* was found to be present in Australia. Close genetic relationships found in populations that were large distances apart (1000 to 2000 km) suggests that seed movement has occurred, which might have been by either wind or animal transport.

Lu *et al*., (2007) working with the self pollinated wind dispersed weed *Lactuca serriola* found that the genotypes of samples collected in the Adelaide region were different to samples collected on the Yorke peninsula, 100 km to the west. Lu et al., (2007) found variation in *L. serriola* genotypes at close distances (<100 km) and that ALS resistant *Lactuca* had moved from the field to the roadside as ALS-inhibiting herbicides were not used on the roadsides. This study found that, although there was evidence of high genetic diversity in *S. oleraceus*, there were similar genotypes separated by large distances (>1200 km). Human mediated movement may have assisted movement of *S. oleraceus* genotypes over large distances, but it is also possible that, unlike *L. serriola*, with its smaller less sophisticated seed and pappus structure, the larger seed and pappus structure of *S. oleraceus* contributes to a greater freedom of movement in the wind. The seed and pappus structure of *S. oleraceus* may contribute to it being picked up by updrafts and carried to high altitude wind currents. Although the actual dispersal method has not been investigated in this study, the results presented here suggest seed movement plays a significant role in the spread of resistance in *S. oleraceus* populations (Chapter 7). Given the ability of *S. oleraceus* seed to spread, resistant seed from adjoining fields, roadsides or railway lines, plants could easily migrate to resistance-free fields.

ALS-inhibiting herbicide resistance to chlorsulfuron in *S. oleraceus* is widespread in Australia. This knowledge in the extent and structure of genetic variation in *S. oleraceus* allows for a greater understanding of the potential for wind dispersed weed populations to disperse. Although human mediated movement can be managed through a variety of practices, such as increased hygiene related to animal and fodder transport, a plant with wind dispersed seed that can be widely dispersed across the landscape will exacerbate managing resistant seed in the landscape. Gene flow of ALS-inhibiting herbicide resistance in *S. oleraceus* is in the main by seed movement and management strategies aimed at reducing the weed seed bank will reduce the level resistant plants.

The finding that a number of different resistant *S. oleraceus* genotypes clusters with distinct specific mutations can exist in a single field provides knowledge for a close understanding of the nature of mutation existence and proliferation by further utilisation of molecular technologies. This finding is shown in chapter 6 with the existence of 2 different known mutations and one unknown mutation or mechanism being found in an individual field. The extinction of resistant genes in a typical field in the southern Australian cropping zone is unlikely to be a practical option (Weersink *et al.*, 2005). This is important as management strategies will need to be aimed at the best economic outcome of reducing the levels of herbicide resistance to a realistic level, given that it is unlikely that total extinction of the resistance allele will occur. Simply zero tolerance may be preferred, but it is unlikely to be economically viable. Farmers will make their compromises to manage at the best economic threshold and future research to understand how the density of *S. oleraceus* impact on productivity would assist these decisions.

Information obtained from research into herbicide resistance can have profound benefits due to the low levels of diversity in Australian crop production and herbicide controls. These aspects have been elucidated by Powles and Shaner (2001), with the authors stating there was a need to educate legislators and farmers about the importance of herbicide mixtures as a strategy to prevent herbicide resistance because once resistant populations spread and increase in density across the Australian landscape eradication is often not economically feasible.

Concerns already exist among weed scientists and agronomists that the current level of adoption of practices that reduce herbicide reliance is sub optimal and that farmers may be unaware of the benefits from preventing herbicide resistance (Weersink 2005). If herbicide resistance is highly mobile between fields and farms there is likely to be less incentive for farmers to invest in preventing resistance (Llewellyn and Allen 2006). Management strategies

to mitigate resistance increasing have been proposed such as using a greater diversity of crops (Broster and Pratley 2008). Diversifying agro-ecosystems and using different herbicide modes of action are being used (Powles and Preston 2006, Powles 2008). This work provides scientific understanding of key aspects of this problem as there is a need to move in this direction in research in addition to crop agronomic relationships (Neve *et al.,* 2009).

Gressel (1982) points out that the pattern of herbicide use by farmers is a significant factor in the evolution of herbicide resistance. The knowledge gained from research is likely to improve the use of herbicides and other management strategies to reduce the increasing problem of herbicide resistance. It is virtually impossible to stop weed seeds migrating by wind into fields however it is possible to rotate herbicide groups, manage rotations and use alternative integrated weed management practices.

FUTURE WORK

The results of this study indicate that ALS-inhibiting herbicide resistance to chlorsulfuron in *S. oleraceus* is now widespread in Australia. The movement of the resistance gene within populations is low (<4%), however, population dendrograms indicate seed has been dispersed across large distances in Australia facilitating the movement of the resistance gene. In addition sequence analysis indicates numerous independent mutation events. Further surveys will enhance the picture of chlorsulfuron resistance levels in *S. oleraceus* in Australia. This study found that collecting individual plant accessions which produced mainly all resistant or all susceptible progeny, elucidates how the frequency of resistance varies in individual fields. More comprehensive individual field collections over a wider area will enhance the picture of the frequency of resistance. Future work could focus on the change in proportion of resistance and susceptible plants over time under different management practices, given we know that gene movement is via seed. Further work may also focus on determining whether populations

of *S. oleraceus* go locally extinct and are then re-populated from neighbouring fields or roadsides and how high density *S. oleraceus* populations affect production levels.

In a broader sphere there are three key events in the environment to study, firstly the selection of resistance, secondly the movement of resistance and finally the extinction of the resistance gene. From this study we now know that *S. oleraceus* has a large genetic diversity and, due to this, it is likely that *S. oleraceus* will readily evolve resistance to many if not all of the herbicide groups known to control it. Managers will therefore need to incorporate numerous integrated management strategies to keep *S. oleraceus* under economical control. For example, since *S. oleraceus* plants resistant to chlorsulfuron were found to be susceptible to imidazolinones then utilizing this herbicide in a farming system will lead to reduced chlorsulfuron resistant plant levels.

Furthermore, utilising the existing seed collection for treatment with other groups of herbicides such as the inhibitors of photosynthesis at photosystem II, inhibitors of tubulin formation, inhibitors of carotenoid biosynthesis and inhibitors of EPSP synthase will clarify the cross resistance picture. Extending the work on the sequence of mutants found in the single field (South 4) in South Australia will identify mutation sites in the unknown resistant plants. Future work to determine changes in the resistance frequency under various management regimes will enhance the improvement of best practice management methods.

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APPENDICES

1. *Sonchus oleraceus* germplasm collection
2. Gene flow experiment data
3. Source of chemicals
4. Source of equipment
5. Oglionucleotides
6. Gels, Buffers and media

Appendix 1. *Sonchus oleraceus* Germplasm

File = BRD801 SonchusQuSedSort50B.XLS

Seed Collection *Sonchus oleraceus* L. (sowthistle).

Robin St.John-Sweeting

Sonchus oleraceus Number So	Date	Location	State	GPS Location UTM or Lat &Long	Status Resistant or Susceptible	Notes
So000100	11 Jun 98	Billa Billa	Qu	28° 10' 00.00"S 150° 16' 00.00"E		
So000101		Projeny of line above	Qu		Res	2R
So000200A	29 May 97	Bowenville 4km West of Bowenville,South of road Near So 124	Qu	27° 16' 50.19"S 151° 27' 15.21"E		
So000200B	29 May 97	Bowenville 4km West of Bowenville,South of road	Qu			
So000300	11 Jul 97	Dalby Ag College old herbicide residue site Near So 125	Qu	27° 08' 00.00"S 151° 17' 11.00"E		
So000400	23 Jul 97	Billa Billa Lapunyah "Green hills" fenceline	Qu			
So000500	11 Jun 98	Croppa Creek Maillers	NSW	27° 07' 32.00"S 150° 18' 26.00"E	Res	5R
So000600	24 Jul 97	North Star "Wind Ridge"	NSW	28° 56' 00"S 150° 24' 00.00"E		
So000700	23 Jul 97	Billa Billa "Te Apiti" road side	Qu			
So000800A	28 Jul 97	Billa Billa Roadside, Millmerran Goondiwindi Rd	Qu	28° 15 ' 59.3"S 150° 23' 20.00"E		
So000800B	28 Jul 97	Billa Billa Roadside, Millmerran Goondiwindi Rd	Qu	28° 15 ' 59.3"S 150° 23' 20.00"E		
So000801		Progeny of line above	Qu			
So000900A	28 Jul 97	North Star Site 2-3km North of North Star Fence line in oats	NSW	28° 52 ' 00.00"S 150° 23' 00.00"E		
So000900B	28 Jul 97	North Star Site 2-3km North of North Star Fence line in oats	NSW	28° 52 ' 00.00"S 150° 23' 00.00"E		
So001000	28 Jul 97	Around tree in wheat paddock Yallaroi 29 09 00.5, 150 28 20	NSW	29° 09 ' 00.50"S 150° 28' 20.00"E		
So001100	28 Jul 97	Fence line at research station 29 18 29.3, 150 28 20 S of Yallaroi	NSW	29° 18 ' 29.30"S 150° 28' 20.00"E		
So001200	28 Jul 97	Showground fence, Kennedy St 30 30 46.9, 151 40 40 Armidale	NSW	30° 30 ' 46.90"S 151° 40' 40.00"E		
So001300	Sep 98	Wallumbilla Tony Bates 'Bengalla' (GR)	Qu	26 35 13 149 11 10		
So001400	4 Aug 97	Dalby Ag. College Hort section	Qu	27 16 00 151 05 69		
So001401		Progeny of line above 14/1 6R in Gene Flow	Qu		Res CT4 P38	6R
So001500	Sep 98	Brigalow	Qu	26° 50' 51.00"S 150° 47' 47.00"E		
So001600	Sep 98	Jackson 'Hillview' (Up Wandsan Rd 10km)	Qu		Res	15R
So001602		Progeny of line above	Qu		Res P38	4R

So001700	6 Aug 97	Gatton College Hort. Section	Qu	27º 33' 34.00"S 152º 16' 46.00"E		
So001800	30 Jul 97	Tamworth Office from gardens adjacent to carpark	NSW	31º 05' 30.00"S 150º 55' 51.00"E		
So001900	4 Sep 97	Toowoomba Gardens at QWRI	Qu	27º 31' 45.00"S 151º 56' 11.00"E		
So002000	6 Oct 98	Dalby Kurralta Park residual herbicide trial	Qu	27 16 00 151 05 69		
So002100	Oct 98	Liverpool Plains Paddock 26 (Lisa Rew)	Qu			
So002200	Oct 98	Croppa Creek CC Field site (Lisa Rew)	NSW			
So002300	Sep 98	Helidon Beside onion field near bypass entrance	Qu			
So002400	6 Oct 98	Dalby Ag College Block A13	Qu	27 16 00 151 05 69		
So002500	Sep 98	Toowoomba Various sites (Nil Herbicide)	Qu	28º 10' 10.00"S 150º 27' 00.00"E		
So002600	25 Aug 98	Billa Billa near JC's lucerne	Qu			
So002700	Sep 98	Condamine 20km south of Miles Near Taylors	Qu			
So002800	Aug 98	Theodore	Qu			
So002900	Aug 98	Banana Johnstone 'Can-berra'	Qu			
So003000	Aug 98	Biloela 'White'	Qu			
So003100	Sep 98	Dulacca 'Stratmore'	Qu			
So003200	25 Aug 98	Aberdeen Turnoff Road side	Qu			
So003300	25 Aug 98	Gore Highway At sign'Mooroondu' 8 km	Qu			
So003400	Sep 98	Roma 14 km East of Roma 'Ironda' (GR)	Qu	26º 34' 51.54"S 148º 57' 27.02"E	Sus	
So003500	Sep 98	Chinchila/Brigalow Rodger 'Cattle camp' (GR)	Qu			
So003600	3 Aug 98	Emerald Research Station (VO, MMc)	Qu			
So003700	Oct 98	Boggabilla Roadside Grey Clay (GR)	Qu			
So003800	Oct 98	North Star Roadside Red Brown Earth (GR)	NSW			
So003900	Sep 98	Esk Potato field Lake Clarendon Road	Qu			
So004000	Sep 98	Gatton Hort Field Section	Qu			
So0041	15 Oct 92	1 bag Seed bag study				
So0042		From outside?				
So0043	Oct 92	Glasshouse				
So0044	7 Sep 92	35 bags				
So0045	1992	1 bag				
So0046	1991	1 bag non selected with ALS inhibitor herbicide in 1991				
So0047	1992	1 bag Glasshouse R to SU's				
So0048		No Seed				
So0049		9A to 9E crosses F2 and F3 Seed				
So0050		10A to 10E crosses R Female X S pollen male				
So0051		Wallumblla 11a to 11F in lucerne				
So0052		12A to 12E crosses S Female x R pollen male				
So0053		No Seed				
So0054		No sSeed				
So0055		Waite	SA			
So0056		Paradise Chris Prestons	SA	34º 52' 24.81"S 138º 40' 08.01"E	Sus	FoodC

So0057	920921	Goondiwindi Res from1991 glasshouse harvest at 4 Degree C	Qu	28º 32' 9.33" S 150º 18' 26.66" E	Res	1R 20R
So0058		From F1 plant number 2 F2 seed backcross to Susept				
So0059		F2 seed backcross toSuscep from number 6 F1 plant				
So0060		F2 seed from F1 backcross to Suscep				
So0061						
So0062		Packet contains 33g of seed plus packet WB23 and WB24				
So0063	1992	Res not selected with ALS Herbc NSW biotype 92 from GHouse				
So0064		Res 4 times 500 seeds				
So0065		F1 backcross to suscep S X R				
So0066		F1 backcross to Suscep R X S				
So0067		Suscep glasshouse harvested spring 1992 47 g				
So0068	10/10/1992	Site 1 Moree Gwydir St				
So0069	10/10/1992	Site 2 Moree Vacant land Edward St				
So0070	10/10/1992	Site 3 Moree Boland drive residential				
So0071	12-10 92	Site 4 Moree Bala St				
So0072	12-10 92	Site 5 Moree Mehi River Collacesbri Rd				
So0101	28 Aug 05	WA050828Rail Wasleys Centre of railway line	SA	34º 28' 51.65"S 138º 41' 12.81"E	Res	18R P7
So0102	27 Aug 05	RO050827N3 Wasleys road by North Paddocks	SA	34º 29' 52.75"S 138º 41' 18.12"E	Res	17R P7
So0103	29 Aug 05	NA050829BFH Nairne Domestic House	SA	35º 02' 42.44"S 138º 54' 43.40"E	Sus	
So0104	27 Sep 05	1 plant Pt Wakefield 1 km N St Kilda TO Oat Pad Playford Sign	SA	34º 42' 54.57"S 138º 34' 35.13"E	Sus	P4
So0105	27 Sep 05	By pump shed soil sterile 2 km N St Kilda TO Pt Wakefield Rd	SA	GR 779 563		
So0106	27 Sep 05	Angle Vale Rd N Side Nr Gawler/Baker Rd Virginia GR776 633	SA	34º 38' 56.29"S 138º 34' 21.94"E	Res	P4 P7
So0107	27 Sep 05	East of Hatchers By centre pivot S of road Tall plant White tip	SA	34º 34' 15.74"S 138º 40' 06.32"E	Sus	P4
So0108	27 Sep 05	Lucas rd Wasleys Rd Kangaroo Flat GR884 742	SA	34º 33' 09.35"S 138º 41' 32.88"E	Res	P4 P7
So0109	27 Sep 05	Centre Wasleys town 5m West of Railway	SA	GR 872 833		
So0110	27 Sep 05	PrattRd Rway 30m N crosng 2m E of rail seams sprayed 10 Sep 05	SA	GR 876 822		
So0111	27 Sep 05	PrattRd RwayX 20m Sof cross 2m west of line seams sprayed10/9/05	SA	GR 876 821		
So0112	27 Sep 05	3 Way Ave Myrtle Bank	SA	GR 844 286		
So0113	27 Sep 05	3 Way Ave Myrtle Bank	SA	GR 844 286		
So0114	28 Sep 05	Adelaide parklands	SA	34º 56' 35.35"S 138º 34' 53.29"E	Sus	
So0115	28 Sep 05	Adelaide parklands	SA	34º 56' 35.35"S 138º 34' 53.29"E	Sus	P4 P7
So0116	29 Sep 05	3 Way Ave Myrtle Bank Yellow Tip Smooth Leaf	SA	GR 844 286		
So0117	29 Sep 05	3 Way Ave Myrtle Bank White Tip Serrate Leaf	SA	GR 844 286		
So0118	30 Sep 05	3 Way ave Myrtle Bank	SA	GR 844 286		
So0119	30 Sep 05	3 Way Ave Myrtle Bank Bright Yellow Flower	SA	GR 844 286		
So0120	6 Oct 05	Waite Institute	SA	34º 58' 06.99"S 138º 38' 02.95"E	Sus	P4
So0121	11 Oct 05	Wasleys House	SA	34º 28' 20.95"S 138º 41' 10.44"E	Sus	P4
So0122	11 Oct 05	Wasleys House	SA		Sus	16R
So0123	18 Oct 05	Oakey next to sorghum field WW Fallow Sorg Sorg Sprayed	Qu	lat 27 24 57 long 151 39 70	Sus	P4

So0124	18 Oct 05	In Sorghum SE of Dalby	Qu	lat 27 18 20 long 151 26 32	Sus	P4
So0125	18 Oct 05	Dalby Ag College One mother plant	Qu	27 08 93 151 17 11	Res	11R P7
So0126	18 Oct 05	Nw of Dalby Edge wheat crop	Qu	27 08 93 151 17 11	Sus	P4
So0127	18 Oct 05	Nw of Dalby In wheat stubble	Qu	27 08 93 151 17 11	Sus	P4
So0128	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 53' 47.59" S 150º 52' 01.49" E	Sus	10R
So0129	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 53' 49.99" S 150º 52' 01.76" E	Sus	
So0130	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 53' 52.97" S 150º 52' 01.77" E	Res	
So0131	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 53' 53.24" S 150º 52' 04.10" E	Res	
So0132	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 53' 53.26" S 150º 52' 06.66" E	Res	
So0133	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 53' 53.14" S 150º 52' 09.01" E	Res	
So0134	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 53' 56.34" S 150º 52' 09.43" E	Res	
So0135	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 53' 59.90" S 150º 52' 11.46" E	Sus	
So0136	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 53' 59.56" S 150º 52' 13.53" E	Sus	
So0137	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 53' 59.08" S 150º 52' 16.02" E	Sus	
So0138	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 54' 01.92" S 150º 52' 16.63" E	Sus	
So0139	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 54' 04.49" S 150º 52' 20.89" E	Sus	
So0140	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 54' 01.89" S 150º 52' 22.90" E	Sus	
So0141	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 53' 59.16" S 150º 52' 24.64" E	Sus	
So0142	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 53' 56.43" S 150º 52' 24.19" E	Sus	
So0143	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 53' 56.63" S 150º 52' 20.68" E	Res	
So0144	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 53' 53.64" S 150º 52' 18.46" E	Sus	
So0145	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 53' 54.76" S 150º 52' 14.65" E	Res	P7
So0146	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 53' 51.49" S 150º 52' 13.99" E	Res	
So0147	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 53' 47.63" S 150º 52' 12.90" E	Res	
So0148	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 53' 47.04" S 150º 52' 07.95" E	Res	
So0149	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 53' 45.71" S 150º 52' 04.74" E	Res	P7
So0150	18 Oct 05	Jason Hughes (Warra/Chinchilla) 1of 23 plants in 25 ha field	Qu	26º 53' 45.49" S 150º 52' 00.04" E	Sus	
So0151	18 Oct 05	Gordon Henry Blackdown W of Condamine	Qu	26 55 35 150 04 64	Res	P4
So0152	18 Oct 05	Russell Topp By Shed near wheat Condamine	Qu	27 06 12 149 56 44	Sus	P4
So0153	18 Oct 05	Russell Topp open area near gate and a shed Condamine	Qu	27 06 12 149 58 17	Sus	P4
So0154	18 Oct 05	Near Gum Russel Topp Not Russel top but W of the Gum	Qu	27 22 08 150 04 71	Res	P4 P7
So0155	18 Oct 05	Just west of Dalby	Qu	27 16 00 151 05 69	Res	P4 P7
So0156	19 Oct 05	West of Brookstead in Barley	Qu	27 47 56 151 24 51	Res	P4 P7
So0157	19 Oct 05	West of Millmeran	Qu	28 00 53 150 53 99	Sus	P4
So0158	19 Oct 05	Ken Campbell Goondiwindi	Qu	28 09 97 150 25 79	Sus	P4
So0159	19 Oct 05	Billa Billa Not spray by tractorTom and Angus Woods propty	Qu	28 10 00 150 27	Res	P4 P7
So0160	19 Oct 05	Billa Billa sprayed 10 days earlier Ally in chick peas	Qu	28 07 61 150 15 29	Sus	P4

So0161	19 Oct 05	Billa Billa by chick peas	Qu	28 07 61 150 15 29	Res	19R P7
So0162	19 Oct 05	Billa Billa not sprayed Fence line centre of property	Qu	28 05 63 150 15 08	Res	P7
So0163	19 Oct 05	Sw of Moonie by wheat paddock	Qu	27 44 13 150 19 80	Res	12R
So0164	19 Oct 05	Moonie oilfield airfield	Qu	27 44 87 150 15 96	Res	P4
So0165	19 Oct 05	By crop wheat Bungunya	Qu	28 01 43 149 41 93	Sus	P4
So0166	19 Oct 05	In chick pea crop with many Sonchus	Qu	28 25 93 149 42 05		
So0167	19 Oct 05	In chick pea crop with many Sonchus	Qu	28 25 93 149 42 05		
So0168	19 Oct 05	In chick pea crop with many Sonchus	Qu	28 25 93 149 42 05	Res	P4 P7
So0169	19 Oct 05	In chick pea crop with many Sonchus	Qu	28 25 93 149 42 05		
So0170	19 Oct 05	In chick pea crop with many Sonchus	Qu	28 25 93 149 42 05	Res	P4 P7
So0171	19 Oct 05	By wheat paddock 10 km E of Goondiwindi by fence	Qu	28 29 84 150 25 08	Res	P4 CT8
So0172	20 Oct 05	5 km North of North Star by wheat crop	NSW	28 52 76 150 23 96	Res	P4
So0173	20 Oct 05	In barley crop betweeen Yallaroi and Croppa creek	NSW	28° 08' 03.00"S 150° 22' 27.00"E	Res	13R
So0174	20 Oct 05	High up at lookout 511m away from cropping over Warialda	NSW	29 29 93 150 34 77	Res	P4
So0175	20 Oct 05	NSW Yetman 1km south No cropping area	NSW	28 55 50 150 46 12	Sus	P4
So0176	20 Oct 05	NSW Yetman 1km south No cropping area	NSW	28 55 50 150 46 12	Res 14R	14R P4
So0177	20 Oct 05	Between Clifton and Nobby just S of Toowoomba **No Seed**	Qu	27 52 39 151 57 64		
So0178	25 Oct 05	Cnr South Tce Hutt St	SA	34 56 05.22 138 36 45.92	Sus	P4
So0179	25 Oct 05	3 Way Ave Myrtle Bank Tall numbered plant 138 38 17.40	SA	GR 844 286 34 57 44.70	Sus	P4
So0180	25 Oct 05	3 Way Ave One Head from one plant	SA	GR 844 286		180
So0181	25 Oct 05	3 Way Ave One Head from one plant	SA	GR 844 286		181
So0182	25 Oct 05	3 Way Ave One Head from one plant	SA	GR 844 286		182
So0183	25 Oct 05	3 Way Ave One Head from one plant	SA	GR 844 286		183
So0184	25 Oct 05	3 Way Ave One Head from one plant	SA	GR 844 286		184
So0185	25 Oct 05	3 Way Ave One Head from one plant	SA	GR 844 286		185
So0186	25 Oct 05	3 Way Ave One Head from one plant	SA	GR 844 286		186
So0187	25 Oct 05	3 Way Ave One Head from one plant	SA	GR 844 286		187
So0188	25 Oct 05	3 Way Ave One Head from one plant	SA	GR 844 286		188
So0189	25 Oct 05	3 Way Ave One Head from one plant	SA	GR 844 286		189
So0190	25 Oct 05	3 Way Ave One Head from one plant	SA	GR 844 286		190
So0191	25 Oct 05	3 Way Ave One Head from one plant	SA	GR 844 286		191
So0192	25 Oct 05	3 Way Ave One Head from one plant	SA	GR 844 286		192
So0193	25 Oct 05	3 Way Ave One Head from one plant	SA	GR 844 286		193
So0194	25 Oct 05	3 Way Ave One Head from one plant	SA	GR 844 286		194
So0195	25 Oct 05	3 Way Ave One Head from one plant White Tip	SA	GR 844 286		195
So0196	25 Oct 05	3 Way Ave One Head from one plant White Tip	SA	34° 57' 44.93"S 138° 38' 17.55"E	Sus 16A	196
So0197	28 Oct 05	63 Birkridge Glenunga	SA		Res 5/20 16B	197
So0198	8 Dec 05	Waite grounds fielsd lab Very white flower	SA	34° 58' 13.55"S 138° 38' 22.89"E	Sus 16A 16B	198
So0199	27 Oct 05	3 Way Short Plant Yellow Flower	SA	GR 844 286	Res 10/40 16A	199
So0200	27 Oct 05	3 Way Yellow	SA	GR 844 286	Sus 16A	200

So0201	27 Oct 05	3 Way Yellow Tall	SA	GR 844 286	Sus 16A	201
So0202	27 Oct 05	3 Way White Tall	SA	GR 844 286		202
So0203	27 Oct 05	3 Way White Tall	SA	GR 844 286		203
So0204	27 Oct 05	3 Way White	SA	GR 844 286		204
So0205	27 Oct 05	3 Way White	SA	GR 844 286		205
So0206	27 Oct 05	3 Way White	SA	GR 844 286		206
So0207	27 Oct 05	3 Way White	SA	GR 844 286		207
So0208	27 Oct 05	3 Way White	SA	GR 844 286		208
So0209	29 Oct 05	3 Way Bulk seed 20,000seeds Mix 40 mother plants	SA	GR 844 286		209
So0210	29 Oct 05	3 Way Burnt pappus largest sieved seed	SA	GR 844 286		210
So0211	29 Oct 05	3 Way Burnt Pappus Mixed 10 heads	SA	GR 844 286		211
So0212	29 Oct 05	3 Way Yellow	SA	GR 844 286		212
So0213	29 Oct 05	3 Way Medium height White tip	SA	GR 844 286		213
So0214	29 Oct 05	3 Way Tall white	SA	GR 844 286		214
So0215	29 Oct 05	3 Way Yellow Tall	SA	GR 844 286	Sus 16A	215
So0216	29-Oct-05	Ridge Park Car Park White tip	SA	34 57 43.01 138 38 25.85	Sus 16A	216
So0217	29-Oct-05	Waite Institute S.asper	SA	34º 58' 06.99"S 138º 38' 02.95"E	Sus 16A	217
So0218	4-Nov-05	In Canola West of Wasleys at corner	SA	34 28 15.33 138 40 41.53		218
So0219	4-Nov-05	InBeans Just N of Mallala turn off to S of campus	SA			219
So0220	4-Nov-05	In Beans just S Wasleys	SA	34 28 41.38 138 40 51.99	Res	220
So0221	4-Nov-05	Wasleys House	SA	34 28 21.04 138 41 12.42		221
So0222	4-Nov-05	Wasleys House	SA			222
So0223	4-Nov-05	Nairne	SA	35 02 42.54 138 54 42.98		223
So0224	3-Nov-05	3 Way white	SA			224
So0225	3-Nov-05	3 Way White	SA			225
So0226	13-Nov-05	Nairne white	SA			226
So0227	13-Nov-05	Nairne White	SA			227
So0228	17-Nov-05	Burnside	SA			228
So0229	17-Nov-05	Burnside	SA			229
So0230	20-Nov-05	Eden Hills Adelaide	SA	35 00 53.33 138 34 54.77	Sus 15B	230
So0231	22-Dec-05	Waite East Dump Many Bright Yellow flowers	SA			231
So0232	22-Dec-05	Waite East Dump	SA			232
So0233	29-Dec-05	Roseworthy Campus East 13 in peas just at harvest	SA			233
So0234	29-Dec-05	Roseworhty Campus East 13 In Peas To Dose Rate expt	SA	34º 30' 10.20"S 138º 41' 24.22"E	Sus	234
So0235	5-Jan-06	Waite Conservation Park S asper	SA			235
So0236	5-Jan-06	Waite Conservation Park S asper	SA			236
So0237	5-Jan-06	Waite Conservation Park S asper	SA			237
So0238	9-Jan-06	Tas Dr Gary Rooke /Tony Cathcart Roaches Beach SE Hobart	Tas	42º 54' 03.12"S 147º 29' 49.23"E	Sus 15B	238
So0239	21-Jan-06	Port Lincoln S asper	SA	34º 44' 46.01"S 135º 52' 45.74"E	Sus 15B	239
So0240	21-Jan-06	Port Lincoln S oleraceus	SA		Sus 15B	240

So0241	21-Jan-05	Port Lincoln S. oleraceus	SA		Sus 15B	241
So0242	21-Jan-05	Port Lincoln S. oleraceus	SA		Sus 15B	242
So0243	Feb 06 ?	From S. Walker and S. Adkins 2/2/06 Qu W2 57A #221 Sus	Qu	26.65272 148.87553	Sus 16A	243
So0244	Feb 06 ?	From S. Walker and S. Adkins 2/2/06 Qu W2 71 #247 Sus	Qu	27.74677 149.71641	Sus 16A	244
So0245	Feb 06 ?	From S. Walker and S. Adkins 2/2/06 Qu W2 62A #232	Qu	27.60215 149.75448	Res 16A	245
So0246	Feb 06 ?	From S. Walker and S. Adkins 2/2/06 Qu W2 80B #259	Qu	27.67376 150.40637	Res 5/40 16A	246
So0247	Feb 06 ?	From S. Walker and S. Adkins 2/2/06 Qu W2 68 #240	Qu	28.12387 149.87428	Res 5/30 16A	247
So0248	Feb 06 ?	From S. Walker and S. Adkins 2/2/06 Qu W2 30 #470 Sus	Qu	26.82071 150.81027	Sus 16A	248
So0249	Feb 06 ?	From S. Walker and S. Adkins 2/2/06 Qu W2 67B #515 Sus	NSW	30.00373 149.64262	Sus 16A	249
So0250	Feb 06 ?	From S. Walker & S. Adkins 2/2/06 Qu Russel Wood #577 **Res**	Qu	26.738 150.618	Res 4/10 16A	250
So0251	Feb 06 ?	From S. Walker and S. Adkins 2/2/06 Qu PM A #578 **Res**	Qu	26.926 150.136	Res 10/40 16A	251
So0252	Feb 06 ?	From S. Walker and S. Adkins 2/2/06 Qu PM D #581 **Res**	Qu	26.926 150.106	Res 15/30 16A	252
So0253	Feb 06 ?	From S. Walker and S. Adkins 2/2/06 Qu W2 21A #173 Sus	Qu	23.86427 148.28670	Sus 16A	253
So0254	05 Dec 05	12 Samp P Boutsalis # 08 54H 5934372N 602302E In beans	VIC		Sus P11	254
So0255	06 Dec 05	12 Samp P Boutsalis #16 54H 5918314N 556962E In wheat	VIC		Sus P11	255
So0256	06 Dec 05	12 Samp P Boutsalis #17 54H 5908633N 555565E In oats	VIC		Sus P11	256
So0257	06 Dec 05	12 Samp P Boutsalis #34 54H 5944955N 631273E In peas	VIC		Sus P11	257
So0258	06 Dec 05	12 Samp P Boutsalis #35 54H 5953601N 632937E In lentils	VIC		Sus P11	258
So0259	07 Dec 05	12 Samp P Boutsalis #38 54H 5955374N 642944E In wheat	VIC		Sus P11	259
So0260	07 Dec 05	12 Samp P Boutsalis#39 54H 5955387N 642988E In chickpeas	VIC		Sus P11	260
So0261	07 Dec 05	12 Samp P Boutsalis #44 54H 5943425N 663584E In lentils	VIC		Sus P11	261
So0262	07 Dec 05	12 Samp P Boutsalis #45 54H 5947158N 667002E In beans	VIC		Sus P11	262
So0263	07 Dec 05	12 Samp P Boutsalis #47 54H 5949850N 684930E In wheat	VIC		Sus P11	263
So0264	07 Dec 05	12 Samp P Boutsalis #113 54H 6072720N 666906E In wheat	VIC		Sus P11	264
So0265	07 Dec 05	12 Samp P Boutsalis #114 54H 6075194N 659700E In wheat	VIC		Res P15B	265
So0266	08 Apr 06	Roseworthy North paddock olive grove	SA	34°30' 43.38"S 138°41' 20.06"E	Sus P15A	266
So0267	08 Apr 06	Roseworthy North paddock olive grove	SA		Sus P15A	267
So0268	08 Apr 06	Roseworthy North paddock olive grove S.asper	SA		Sus	268
So0269	31 May 06	Roseworthy S of main build in stubble field S of stock rate trial	SA	34°32' 24.31"S 138°41' 29.26"E	Sus P15A	269
So0270	31 May 06	Wasleys road next to Faba bean field of 2005 close to So220	SA	34 28 40 138 40 51	Res 15B	270
So0271	23 Jun 06	Wasleys West end Annie Tce By 2005 Canola	SA	34 28 16 138 40 41	Res 15B	271
So0272	11 Jul 06	Twartz rd Gawler By crossing rail	SA	34°34' 02.62"S 138°43' 37.17"E	Sus 15B	272
So0273	29 Jul 06	SE corner of E6 Roseworthy Campus Edge of Graingers road	SA	34°30' 54.50"S 138°41' 55.00"E	Res P15B	P4 P12
So0274	29 Jul 06	SE corner of E7 Roseworthy Campus Edge of Graingers road	SA	34°30' 58.11"S 138°42' 34.10"E	Sus	P4
So0275	23 Mar 06	Nairne	SA	35° 02 ' 42.54"S 138° 54' 42.98"E	Sus 14B	P4
So0276	26 Aug 06	Semaphore Blackler St in sand garden 6 heads Plenty Seed	SA	35° 50 ' 22.22"S 138° 28' 52.11"E	Sus 14B	276
So0277	14 Sep 06	Olives north Roseworthy Campus Row 1 approx 10 heads	SA	34 30 ' 47.65"S 138° 41' 22.10"E	Sus 15A	277
So0278	14 Sep 06	Olives north Roseworthy Campus Row 5 approx 06 heads	SA	34° 30 ' 46.82"S 138° 41' 21.89"E	Sus 15A	278
So0279	14 Sep 06	Olives north Roseworthy Campus Row 10 approx 08 heads	SA	34° 30 ' 45.72"S 138° 41' 21.70"E	Sus 15A	279

So0280	14 Sep 06	Olives north Roseworthy Campus Row 15 approx 20 heads	SA	34° 30 ' 44.35"S 138° 41' 21.77"E	Sus 15A	280
So0281	14 Sep 06	Olives north Roseworthy Campus Row 20 approx 08 heads	SA	34° 30 ' 43.24"S 138° 41' 21.53"E	Sus 15A	281
So0282	14 Sep 06	Olives north Roseworthy Campus Row 25 approx 10 heads	SA	34° 30 ' 42.32"S 138° 41' 21.46"E	Sus 15A	282
So0283	14 Sep 06	Olives north Roseworthy Campus Row 30 approx 10 heads	SA	34° 30 ' 41.04"S 138° 41' 21.87"E	Sus 15A	283
So0284	14 Sep 06	Olives north Roseworthy Campus Row 35 approx 10 heads	SA	34° 30 ' 39.84"S 138° 41' 21.89"E	Sus 15A	284
So0285	12 Oct 06	Baratta Hill East billabong pool N edge in reedsThur 11.00 am	SA	31° 56 ' 09.54"S 139° 05'44.64 E	Sus 14B	285
So0286	02 Nov 06	RAC S4 01	SA	34° 33 ' 05.00"S 138° 41'32.00 E	Sus Conf	286
So0287	02 Nov 06	RAC S4 02	SA	34° 33 ' 02.50"S 138° 41' 30.00"E	Sus P8 CT9	287
So0288	2 Nov 06	RAC S4 03 R4	SA	34° 33 ' 02.50"S 138° 41' 32.50"E	Res P8 CT9	288
So0289	2 Nov 06	RAC S4 04 R3	SA	34° 33 ' 00.00"S 138° 41' 32.50"E	Res P8 CT9	289
So0290	2 Nov 06	RAC S4 05	SA	34° 33 ' 00.00"S 138° 41' 30.00"E	Sus P8 CT9	290
So0291	2 Nov 06	RAC S4 06 R3	SA	34° 33 ' 00.00"S 138° 41' 27.50"E	Res P8 CT9	291
So0292	2 Nov 06	RAC S4 07	SA	34° 32 ' 57.50"S 138° 41' 27.50"E	Sus P8 CT9	292
So0293	2 Nov 06	RAC S4 08	SA	34° 32 ' 57.50"S 138° 41' 30.00"E	Sus P8 CT9	293
So0294	2 Nov 06	RAC S4 09	SA	34° 32 ' 57.50"S 138° 41' 32.50"E	Sus P8 CT9	294
So0295	2 Nov 06	RAC S4 10 R4	SA	34° 32 ' 55.00"S 138° 41' 32.50"E	Res P8 CT9	295
So0296	2 Nov 06	RAC S4 11	SA	34° 32 ' 55.00"S 138° 41' 30.00"E	Sus P8 CT9	296
So0297	2 Nov 06	RAC S4 12	SA	34° 32 ' 55.00"S 138° 41' 27.50"E	Sus P8 CT9	297
So0298	2 Nov 06	RAC S4 13	SA	34° 32 ' 55.00"S 138° 41' 25.00"E	Sus P8 CT9	298
So0299	2 Nov 06	RAC S4 14	SA	34° 32 ' 52.50"S 138° 41' 22.50"E	Sus P8 CT9	299
So0300	2 Nov 06	RAC S4 15	SA	34° 32 ' 52.50"S 138° 41' 25.00"E	Sus P8 CT9	300
So0301	2 Nov 06	RAC S4 16	SA	34° 32 ' 52.50"S 138° 41' 27.50"E	Sus P8 CT9	301
So0302	2 Nov 06	RAC S4 17 R1	SA	34° 32 ' 52.50"S 138° 41' 30.00"E	Res P8 CT9	302
So0303	2 Nov 06	RAC S4 18 R4	SA	34° 32 ' 52.50"S 138° 41' 32.50"E	Res P8 CT9	303
So0304	2 Nov 06	RAC S4 19 R1	SA	34° 32 ' 50.00"S 138° 41' 32.50"E	Res P8 CT9	304
So0305	2 Nov 06	RAC S4 20	SA	34° 32 ' 50.00"S 138° 41' 30.00"E	Sus P8 CT9	305
So0306	2 Nov 06	RAC S4 21	SA	34° 32 ' 50.00"S 138° 41' 27.50"E	Sus P8 CT9	306
So0307	2 Nov 06	RAC S4 22	SA	34° 32 ' 50.00"S 138° 41' 25.00"E	Sus P8 CT9	307
So0308	2 Nov 06	RAC S4 23 R3	SA	34° 32 ' 50.00"S 138° 41' 22.50"E	Res P8 CT9	308
So0309	2 Nov 06	RAC S4 24 R2	SA	34° 32 ' 50.00"S 138° 41' 20.00"E	Res P8 CT9	309
So0310	2 Nov 06	RAC S4 25 R1	SA	34° 32 ' 47.50"S 138° 41' 17.50"E	Res P8 CT9	310
So0311	2 Nov 06	RAC S4 26 R2	SA	34° 32 ' 47.50"S 138° 41' 20.00"E	Res P8 CT9	311
So0312	2 Nov 06	RAC S4 27	SA	34° 32 ' 47.50"S 138° 41' 22.50"E	Sus P8 CT9	312
So0313	2 Nov 06	RAC S4 28 R2	SA	34° 32 ' 47.50"S 138° 41' 25.00"E	Res P8 CT9	313
So0314	2 Nov 06	RAC S4 29 R1	SA	34° 32 ' 47.50"S 138° 41' 27.50"E	Res P8 CT9	314

So0315	2 Nov 06	RAC S4 30	SA	34° 32 ' 47.50"S 138° 41' 30.00"E	Sus P8 CT9	315
So0316	2 Nov 06	RAC S4 31	SA	34° 32 ' 45.00"S 138° 41' 30.00"E	Sus P8 CT9	316
So0317	2 Nov 06	RAC S4 32	SA	34° 32 ' 45.00"S 138° 41' 27.50"E	Sus P8 CT9	317
So0318	2 Nov 06	RAC S4 33	SA	34° 32 ' 45.00"S 138° 41' 25.00"E	Sus P8 CT9	318
So0319	2 Nov 06	RAC S4 34 R1	SA	34° 32 ' 45.00"S 138° 41' 22.50"E	Res P8 CT10	319
So0320	2 Nov 06	RAC S4 35 R1	SA	34° 32 ' 45.00"S 138° 41' 20.00"E	Res P8 CT10	320
So0321	2 Nov 06	RAC S4 36	SA	34° 32 ' 45.00"S 138° 41' 17.50"E	Sus P8 CT10	321
So0322	2 Nov 06	RAC S4 37	SA	34° 32 ' 45.00"S 138° 41' 15.00"E	Sus P8 CT10	322
So0323	2 Nov 06	RACNorth of East 2 by fence	SA	34° 31' 42.00"S 138° 41' 28.00"E	Sus P14B	323
So0324	2 Nov 06	RAC in Grazing Trial	SA	34° 31' 38.00"S 138° 41' 35.00"E	Sus P14B	324
So0325	2 Nov 06	RAC By Labs	SA	34° 31' 42.00"S 138° 41' 11.00"E	Sus P14B	325
So0326	2 Nov 06	RAC By chem lab	SA	34° 31' 42.00"S 138° 41' 11.00"E	Sus P14B	326
So0327	13 Nov 06	Stirling	SA	35° 00' 14.69"S 138° 42' 54.92"E	Res P14B	327
So0328	13 Nov 06	Stirling	SA	35° 00' 54.42"S 138° 42' 52.82"E	Sus P14B	328
So0329	02 Nov 06	RAC E1 01 11 Heads In Peas Peas harvest 3.25pm 2 Nov 06	SA	34° 31' 54" S 138° 41' 30" E	Sus P11	329
So0330	02 Nov 06	RAC E1 02 Large Plant 100 head 4 head harvest	SA	34° 31' 53" S 138° 41' 31" E	Sus P11	330
So0331	02 Nov 06	RAC E1 03 Large plant 200 heads 18 open collect Plenty seed	SA	34° 31' 55" S 138° 41' 34" E	Sus P11	331
So0332	02 Nov 06	RAC E1 04 12 heads collected large plant	SA	34° 31' 57" S 138° 41' 38" E	Sus P11	332
So0333	02 Nov 06	RAC E1 05 Tall outstanding plant 80 heads 4 collected	SA	34° 31' 58" S 138° 41' 42" E	1/9 Res P11	333
So0334	02 Nov 06	RAC E1 06 Large plant 100 heads Red leaves check next year	SA	34° 31' 59" S 138° 41' 44" E	Sus P11	334
So0335	02 Nov 06	RAC E1 07 Lge plant 200 heads 10 heads collect seed 10m	SA	34° 31' 59" S 138° 41' 45" E	Sus P11	335
So0336	02 Nov 06	RAC E1 08 Post at east marked with R 60 heads on East fence	SA	34° 31' 59" S 138° 41' 48" E	1/9Res P11	336
So0337	02 Nov 06	RAC E1 09 Medium plant 40 heads 3 collected	SA	34° 32' 01" S 138° 41' 39" E	Sus P11	337
So0338	02 Nov 06	RAC E1 10 150 heads large plant 10 heads harvested	SA	34° 32' 00" S 138° 41' 33" E	Sus P11	338
So0339	02 Nov 06	RAC E1 11 1 large plant	SA	34° 31' 58" S 138° 41' 30" E	Sus P11	339
So0340	21 Nov 06	Warra Bridge Near Jason Hughes	Qu	26° 55' 41" S 150° 55' 02" E	Sus Planting 10	340
So0341	21 Nov 06	Chinchilla Weir	Qu	26° 48' 00" S 150° 34' 37" E	Sus P20 P10	341
So0342	21 Nov 06	01 Taylor Wheat stubble S of Condamine 12 sample in field	Qu	27° 06' 27" S 150° 08' 31" E	1/7Res P20 P10	342
So0343	21 Nov 06	02 Taylor Glad and Sue Taylor (07) 4669 2176	Qu	27° 06' 24" S 150° 08' 29" E	2/9Res P10 P20	CT11
So0344	21 Nov 06	03 Taylor Glad and Sue Taylor Culara Condamine 4416 Aust	Qu	27° 06' 23" S 150° 00' 27" E	3/9Res P10 P20	CT11
So0345	21 Nov 06	04 Taylor Large plant with plent of seed number 4	Qu	27° 06' 22" S 150° 08' 27" E	2/9Res P10 P20	CT11
So0346	21 Nov 06	05 Taylor	Qu	27° 06' 21" S 150° 08' 22" E	5/9Res P10 P20	CT11
So0347	21 Nov 06	06 Taylor	Qu	27° 06' 22" S 150° 08' 32" E	4/9Res P10 P20	CT11
So0348	21 Nov 06	07 Taylor	Qu	27° 06' 19" S 150° 08' 34" E	3/9Res P10 P20	CT11
So0349	21 Nov 06	08 Taylor	Qu	27° 06' 22" S 150° 08' 32" E	1/2Res P10 P20	CT11

So0350	21 Nov 06	09 Taylor	Qu	27º 06' 22" S	150º 08' 32" E	Res P10 P20	350
So0351	21 Nov 06	10 Taylor	Qu	27º 06' 23" S	150º 00' 33" E	Res 1/9 CT11	351
So0352	21 Nov 06	11 Taylor	Qu	27º 06' 27" S	150º 08' 31" E	Sus CT 11	352
So0353	21 Nov 06	12 Taylor	Qu	27º 06' 29" S	150º 08' 33" E	Res 2/8 CT11	353
So0354	22 Nov 06	01 Brookstead edge of sorghum stubble 5 samples	Qu	27º 45' 30" S	151º 28' 56" E	No Plant	354
So0355	22 Nov 06	02 Brookstead edge of sorghum stubble	Qu	27º 45' 31" S	151º 28' 57" E	No Plant	355
So0356	22 Nov 06	03 Brookstead edge of sorghum stubble	Qu	27º 45' 30" S	151º 28' 56" E	Res 1/9 Ct11	356
So0357	22 Nov 06	04 Brookstead edge of sorghum stubble	Qu	27º 45' 31" S	151º 28' 55" E	No Plant	357
So0358	22 Nov 06	05 Brookstead edge of sorghum stubble	Qu	27º 45' 31" S	151º 28' 54" E	1/9Res	358
So0359	22 Nov 06	01 Pampas roadside by cotton stubble 5 samples	Qu	27º 47' 33" S	151º 24' 15" E	No Plant	359
So0360	22 Nov 06	02 Pampas roadside by cotton stubble 5 samples	Qu	27º 47' 33" S	151º 24' 15" E	No Plant	360
So0361	22 Nov 06	03 Pampas roadside by cotton stubble 5 samples	Qu	27º 47' 33" S	151º 24' 16" E	No Plant	361
So0362	22 Nov 06	04 Pampas roadside by cotton stubble 5 samples	Qu	27º 47' 33" S	151º 24' 16" E	No Plant	362
So0363	22 Nov 06	05 Pampas roadside by cotton stubble 5 samples	Qu	27º 47' 34" S	151º 24' 15" E	No Plant	363
So0364	22 Nov 06	01 09.55am Sorghum stubble in paddock black earth LH side	Qu	27º 49' 51" S	151º 20' 54" E	2/9Res P10 CT12	364
So0365	22 Nov 06	02 09.56am Sorghum stubble in paddock black earth LH side	Qu	27º 49' 52" S	151º 20' 56" E	1/7Res P10 CT12	365
So0366	22 Nov 06	03 09.57am Sorghum stubble in paddock black earth LH side	Qu	27º 49' 54" S	151º 20' 58" E	No Plant	366
So0367	22 Nov 06	04 09.58am Sorghum stubble in paddock black earth LH side	Qu	27º 49' 54" S	151º 20' 57" E	No Plant	367
So0368	22 Nov 06	05 09.59am Sorghum stubble in paddock black earth LH side	Qu	27º 49' 54" S	151º 20' 54" E	No Plant	368
So0369	22 Nov 06	11.45am Billa roadside 3 open head 1 closed Kilbronae	Qu	28º 15' 48" S	150º 23' 17" E	2/9Res P10	369
So0370	22 Nov 06	Carpendale (Andrew) Cattle yard 10 heads	Qu	28º 17' 54" S	150º 22' 19" E	P10 Ct12	370
So0371	22 Nov 06	01 Carpendale wheat stubble just sprayed 10 samples	Qu	28º 16' 57" S	150º 22' 56" E	CT12	371
So0372	22 Nov 06	02 Carpendale Andrew	Qu	28º 16' 56" S	150º 22' 59" E	2/6Res P10 CT12	372
So0373	22 Nov 06	03 Carpendale Post to Gore Highway Goondiwindi 4390	Qu	28º 16' 56" S	150º 22' 59" E	Sus P10	373
So0374	22 Nov 06	04 Carpendale	Qu	28º 16' 57" S	150º 22' 59" E	3/9ResP10 CT12	374
So0375	22 Nov 06	05 Carpendale	Qu	28º 16' 57" S	150º 22' 59" E	Res P10	375
So0376	22 Nov 06	06 Carpendale	Qu	28º 16' 57" S	150º 23' 01" E	Sus P10	376
So0377	22 Nov 06	07 Carpendale	Qu	28º 16' 58" S	150º 23' 02" E	1/9Res P10	377
So0378	22 Nov 06	08 Carpendale	Qu	28º 16' 58" S	150º 23' 02" E	Sus P10	378
So0379	22 Nov 06	09 Carpendale	Qu	28º 16' 58" S	150º 23' 03" E	No Plant	379
So0380	22 Nov 06	10 Carpendale	Qu	28º 16' 58" S	150º 23' 05" E		380
So0381	22 Nov 06	01 South of Carpendale 04.40pm Paddock of wheat stubble	Qu	28º 18' 30" S	150º 21' 54" E		381
So0382	22 Nov 06	02 Fence edge	Qu	28º 18' 30" S	150º 21' 54" E		382
So0383	22 Nov 06	03 Fence edge	Qu	28º 18' 29" S	150º 21' 55" E		383
So0384	22 Nov 06	04 In paddock wheat stubble	Qu	28º 18' 30" S	150º 21' 55" E		384

So0385	22 Nov 06	5	Qu	28° 18' 30" S 150° 21' 55" E		385
So0386	22 Nov 06	6	Qu	28° 18' 31" S 150° 21' 55" E		386
So0387	22 Nov 06	7	Qu	28° 18' 33" S 150° 21' 49" E		387
So0388	22 Nov 06	8	Qu	28° 18' 32" S 150° 21' 49" E		388
So0389	22 Nov 06	9	Qu	28° 18' 31" S 150° 21' 49" E		389
So0390	22 Nov 06	10 In paddock wheat stubble 10 of 10	Qu	28° 18' 30" S 150° 21' 50" E		390
So0391	23 Nov 06	01 at 09.25am Roadside between 2 wheat stubbles 1 of 5 plants	NSW	28° 48' 49" S 150° 25' 03" E	Res CT8	391
So0392	23 Nov 06	02 at 09.27am Tall large plant with plenty of seed to disperse	NSW	28° 48' 49" S 150° 25' 02" E		392
So0393	23 Nov 06	03 at 09.28am Roadside between 2 wheat stubbles	NSW	28° 48' 48" S 150° 25' 03" E		393
So0394	23 Nov 06	04 at 09.30am Roadside between 2 wheat stubbles	NSW	28° 48' 48" S 150° 25' 04" E		394
So0395	23 Nov 06	05 at 09.31am Roadside between 2 wheat stubbles	NSW	28° 48' 48" S 150° 25' 04" E		395
So0396	23 Nov 06	10.05am edge wheat crop Caltrop high level	NSW	28° 52' 13.00"S 150° 24' 07.00"E		396
So0397	23 Nov 06	10.07am edge wheat crop	NSW	28° 52' 14.00"S 150° 24' 07.00"E	Sus	397
So0398Medic	23 Nov 06	10.12am *Medicago*On rail line maybe ALS resistant? V Dry	NSW	28° 52' 16.00"S 150° 24' 07.00"E		398
So0399	23 Nov 06	10.13am	NSW	28° 52' 14.00"S 150° 24' 07.00"E		399
So0400	23 Nov 06	10.19am Fence line Wheat stubble near shed Little in Field	NSW	28° 53' 01.00"S 150° 23' 59.00"E		400
So0401	23 Nov 06	10.20am Fence line Plant with lot of seed Track progeny	NSW	28° 53' 01.00"S 150° 23' 59.00"E	Res CT 15	401
So0402	23 Nov 06	10.21am Fence line Plant with lot of seed 6 heads	NSW	28° 53' 02.00"S 150° 23' 58.00"E	Res CT 15 B3	402
So0403	23 Nov 06	10.23am Fence line Plant with lot of seed 5 heads	NSW	28° 53' 03.00"S 150° 23' 58.00"E	Res CT 15	403
So0404	23 Nov 06	10.24am Fence line Plant with lot of seed 6 heads	NSW	28° 53' 04.00"S 150° 23' 58.00"E	Res CT 15	404
So0405	23 Nov 06	10.25am Fence line Plant with lot of seed 6 heads	NSW	28° 53' 04.00"S 150° 23' 58.00"E	Res CT 15	405
So0406	23 Nov 06	10.34am Fence line Wheat stubble with no Sonchus	NSW	28° 54' 47.00"S 150° 23' 40.00"E	Res CT 15	406
So0407	23 Nov 06	10.36am	NSW	28° 54' 48.00"S 150° 23' 40.00"E	Res CT 15	407
So0408	23 Nov 06	10.37am V L:arge plant High seed Track progeny	NSW	28° 54' 49.00"S 150° 23' 40.00"E	Res CT 15 H2	408
So0409	23 Nov 06	10.40am	NSW	28° 54' 50.00"S 150° 23' 40.00"E	Res CT 15	409
So0410	23 Nov 06	10.41am	NSW	28° 54' 50.00"S 150° 23' 40.00"E	Res CT 15	410
So0411	23 Nov 06	NL 10.48am Fence Line by clear of Sonchus wheat stubble	NSW	28° 56' 36.00"S 150° 24' 03.00"E	Res CT 15	411
So0412	23 Nov 06	NL 10.52am Fence Line by clear of Sonchus wheat stubble	NSW	28° 56' 28.00"S 150° 24' 05.00"E	Res CT 15	412
So0413	23 Nov 06	NL 10.53am Fence Line by clear of Sonchus wheat stubble	NSW	28° 56' 27.00"S 150° 24' 05.00"E	Sus CT15	413
So0414	23 Nov 06	NL 10.54am Fence Line by clear of Sonchus wheat stubble	NSW	28° 56' 26.00"S 150° 24' 05.00"E	Sus CT15 N	414
So0415	23 Nov 06	NL 10.55am Fence Line Large plant Plenty seed	NSW	28° 56' 25.00"S 150° 24' 05.00"E	Sus CT15 O	415
So0416	23 Nov 06	NL 10.57am Fence Line by clear of Sonchus wheat stubble	NSW	28° 56' 23.00"S 150° 24' 05.00"E	Res CT 15 P	416
So0417	23 Nov 06	11.07am Fence line by wheat stubble	NSW	28° 55' 53.00"S 150° 22' 50.00"E	Res CT 15 Q	417
So0418	23 Nov 06	11.09am In gap in wheat stubble	NSW	28° 55' 54.00"S 150° 22' 49.00"E	Res CT 15 R	418
So0419	23 Nov 06	11.10am In wheat stubble	NSW	28° 55' 54.00"S 150° 22' 49.00"E	Res CT 15 S	419

So0420	23 Nov 06	11.11am Fence line by wheat stubble Small plant	NSW	28º 55' 53.00"S 150º 22' 48.00"E	Res CT 15 T	420
So0421	23 Nov 06	11.12am Fence line by wheat stubble Short prolific plant	NSW	28º 55' 53.00"S 150º 22' 49.00"E	Res P14A	421
So0422	23 Nov 06	11.14am Fence line by wheat stubble	NSW	28º 55' 53.00"S 150º 22' 49.00"E	Res P14A	422
So0423	23 Nov 06	2.00pm Yarril Creek	Qu	28º 18' 00.00"S 150º 17' 42.00"E	Res P14A	423
So0424	23 Nov 06	2.05pm Yarril Ck on Bridge West of Wyaga Billa Goondiwindi	Qu	28º 18' 00.00"S 150º 17' 41.00"E	Sus P14A	424
So0425	23 Nov 06	2.06pm Yarril Ck on bridge	Qu	28º 18' 00.00"S 150º 17' 41.00"E	Res P14A	425
So0426	23 Nov 06	NL 2.45pm	Qu	28º 11' 21.00"S 150º 27' 17.00"E	Res P14A	426
So0427	23 Nov 06	Nl 2.46pm	Qu	28º 11' 21.00"S 150º 27' 17.00"E	Res P14A	427
So0428	20 Nov 06	Toowooomba Centre Whole plant	Qu	28º 53' 01.00"S 150º 23' 59.00"E	Res P14A	428
So0429	20 Nov 06	Toowoomba Centre Car Park	Qu	28º 53' 01.00"S 150º 23' 59.00"E	Sus P14A	429
So0430	20 Nov 06	Toowoomba Centre Car Park	Qu	28º 53' 01.00"S 150º 23' 59.00"E		430
So0431	21 Nov 06	Toowoomba Town opposite Allan Cunningham Motel	Qu	27º 35' 18" S 151º 56' 54" E	Sus P14A	431
So0432	31 Mar 07	East of South 4 RAC by roadside SE of S4 road intersection	SA	34º 33 ' 12.00"S 138º 41' 39.00"E		
So0433	31 Mar 07	East of South 4 RAC by roadside	SA	34º 33 ' 01.00"S 138º 41' 35.30"E		
So0434	31 Mar 07	East of South 4 RAC by roadside	SA	34º 32 ' 56.20"S 138º 41' 35.00"E		
So0435	31 Mar 07	East of South 4 RAC by roadside In line centre pivot	SA	34º 32 ' 52.45"S 138º 41' 34.81"E		
So0436	31 Mar 07	East of South 4 RAC by roadside	SA	34º 32 ' 54.71"S 138º 41' 34.26"E		
So0437	31 Mar 07	East of South 4 RAC by roadside	SA	34º 32 ' 54.02"S 138º 41' 34.85"E		
So0438	31 Mar 07	East of South 4 RAC by roadside	SA	34º 32 ' 54.65"S 138º 41' 34.87"E		
So0439	31 Mar 07	East of South 4 RAC by roadside	SA	34º 32 ' 55.24"S 138º 41' 34.90"E		
So0440	31 Mar 07	East of South 4 RAC by roadside	SA	34º 32 ' 45.05"S 138º 41' 34.00"E		
So0441	31 Mar 07	East of South 4 RAC by roadside Near Mallalla turn off	SA	34º 32 ' 46.02"S 138º 41' 34.11"E		
So0442	22 May 07	W of Roseworhty Town Mackereth Road Roseworthy GR 91 79	SA	34º 30 ' 33.36"S 138º 43' 49.24"E		
So0443	22 May 07	W of Roseworthy Town	SA	34º 30 ' 31.69"S 138º 43' 49.67"E		
So0444	22 May 07	W of Roseworthy Town	SA	34º 30 ' 29.68"S 138º 43' 49.61"E		
So0445	22 May 07	W of Roseworthy Town	SA	34º 30 ' 03.92"S 138º 43' 53.53"E		
So0446	22 May 07	W of Roseworthy Town	SA	34º 30 ' 05.74"S 138º 43' 54.31"E		
So0447	22 May 07	W of Roseworthy Town	SA	34º 29 ' 17.32"S 138º 42' 00.03"E		
So0448	22 May 07	W of Roseworthy Town	SA	34º 30 ' 24.20"S 138º 43' 03.70"E		
So0449	22 May 07	W of Roseworthy Town	SA	34º 30 ' 24.21"S 138º 43' 02.61"E		
So0450	22 May 07	W of Roseworthy Town Bill Fennescey 0418 851 483 1.25pm	SA	34º 30 ' 25.43"S 138º 43' 32.02"E		
So0451	04 Jun 07	S of Pengelly Scrub	SA	34º 30 ' 36.81"S 138º 42' 11.57"E		
So0452	04 Jun 07	S of Pengelly Scrub	SA	34º 31 ' 34.56"S 138º 42' 30.76"E		
So0453	4 Jun 07	S of Pengelly Scrub	SA	34º 31 ' 35.38"S 138º 42' 30.67"E		
So0454	4 Jun 07	S of Pengelly Scrub	SA	34º 31 ' 39.15"S 138º 42' 30.35"E		

So0455	4 Jun 07	S of Pengelly Scrub	SA	34º 31 ' 55.05"S 138º 42' 27.12"E		
So0456	4 Jun 07	S of Pengelly Scrub	SA	34º 31 ' 57.19"S 138º 42' 26.80"E		
So0457	4 Jun 07	S of Pengelly Scrub	SA	34º 33 ' 12.02"S 138º 41' 53.11"E		
So0458	4 Jun 07	S of Pengelly Scrub	SA	34º 33 ' 12.07"S 138º 41' 53.38"E		
So0459	4 Jun 07	S of Pengelly Scrub	SA	34º 33 ' 14.61"S 138º 42' 14.76"E		
So0460	4 Jun 07	S of Pengelly Scrub	SA	34º 33 ' 14.58"S 138º 42' 15.12"E		
So0461	4 Jun 07	S of Pengelly Scrub	SA	34º 33 ' 18.95"S 138º 43' 05.41"E		
So0462	4 Jun 07	S of Pengelly Scrub	SA	34º 33 ' 27.73"S 138º 43' 23.33"E		
So0463	9 May 07	St Kilda to Roseworthy Location 1 Plant 1	SA	34º 42 ' 59.00"S 138º 32' 28.00"E		
So0464	9 May 07	St Kilda to Roseworthy Location 1 Plant 2	SA	34º 42 ' 59.00"S 138º 32' 28.00"E		
So0465	9 May 07	St Kilda to Roseworthy Location 1 Plant 3	SA	34º 42 ' 59.00"S 138º 32' 28.00"E		
So0466	9 May 07	St Kilda to Roseworthy Location 1 Plant 4	SA	34º 42 ' 59.00"S 138º 32' 28.00"E		
So0467	9 May 07	St Kilda to Roseworthy Location 1 Plant 5	SA	34º 42 ' 59.00"S 138º 32' 28.00"E		
So0468	9 May 07	St Kilda to Roseworthy Location 1 Plant 6	SA	34º 42 ' 59.00"S 138º 32' 28.00"E		
So0469	9 May 07	St Kilda to Roseworthy Location 2 Plant 7	SA	34º 41 ' 27.00"S 138º 31' 45.00"E		
So0470	9 May 07	St Kilda to Roseworthy Location 2 Plant 8	SA	34º 41 ' 27.00"S 138º 31' 45.00"E		
So0471	9 May 07	St Kilda to Roseworthy Location 2 Plant 9	SA	34º 41 ' 27.00"S 138º 31' 45.00"E		
So0472	9 May 07	St Kilda to Roseworthy Location 2 Plant 10	SA	34º 41 ' 27.00"S 138º 31' 45.00"E		
So0473	9 May 07	St Kilda to Roseworthy Location 2 Plant 11	SA	34º 41 ' 27.00"S 138º 31' 45.00"E		
So0474	9 May 07	St Kilda to Roseworthy Location 2 Plant 12	SA	34º 41 ' 27.00"S 138º 31' 45.00"E		
So0475	9 May 07	St Kilda to Roseworthy Location 3 Plant 13	SA	34º 40 ' 04.00"S 138º 31' 18.00"E		
So0476	9 May 07	St Kilda to Roseworthy Location 3 Plant 14	SA	34º 40 ' 04.00"S 138º 31' 18.00"E		
So0477	9 May 07	St Kilda to Roseworthy Location 3 Plant 15	SA	34º 40 ' 04.00"S 138º 31' 18.00"E		
So0478	9 May 07	St Kilda to Roseworthy Location 4 Plant 16	SA	34º 40 ' 03.00"S 138º 31' 20.00"E		
So0479	10 May 07	Roadside East of S4 RAC 2pm UTM 54 02 882 00 61 746 41	SA	34º 32 ' 53"S 138º 41' 32"E		
So0480	10 May 07	Roadside East of S4 RAC 2.04pm	SA	34º 32 ' 54"S 138º 41' 32"E		
So0481	10 May 07	Roadside East of S4 RAC 2.05pm	SA	34º 32 ' 54"S 138º 41' 32"E		
So0482	10 May 07	Roadside East of S4 RAC 2.07pm	SA	34º 32 ' 54"S 138º 41' 30"E		
So0483	10 May 07	Roadside East of S4 RAC 2.08pm	SA	34º 32 ' 54"S 138º 41' 28"E		
So0484	10 May 07	Roadside East of S4 RAC 2.10pm	SA	34º 32 ' 55"S 138º 41' 25"E		
So0485	10 May 07	Roadside East of S4 RAC 2.11pm	SA	34º 32 ' 54"S 138º 41' 24"E		
So0486	10 May 07	Roadside East of S4 RAC 2.14pm	SA	34º 32 ' 52"S 138º 41' 23"E		
So0487	10 May 07	Roadside East of S4 RAC 2.15pm	SA	34º 32 ' 51"S 138º 41' 24"E	P31	
So0488	10 May 07	Roadside East of S4 RAC 2.16pm	SA	34º 32 ' 50"S 138º 41' 25"E	P31	
So0489	10 May 07	Roadside East of S4 RAC 2.18pm	SA	34º 32 ' 49"S 138º 41' 26"E	P31	

So0490	10 May 07	Roadside East of S4 RAC 2.20pm	SA	34º 32 ' 46"S 138º 41' 28"E		
So0491	9 Jun 07	S of S4 RAC Roadside no ALS sprayed soursob present	SA	34º 33 ' 50"S 138º 41' 38"E		
So0492	9 Jun 07	S of S4 RAC Roadside no ALS sprayed soursob present	SA	34º 33 ' 50"S 138º 41' 38"E		
So0493	9 Jun 07	S of S4 RAC Roadside no ALS sprayed soursob present	SA	34º 33 ' 23"S 138º 41' 35"E		
So0494	9 Jun 07	S of S4 RAC Roadside no ALS sprayed soursob present	SA	34º 33 ' 23"S 138º 41' 35"E		
So0495	9 Jun 07	S of S4 RAC Roadside no ALS sprayed soursob present	SA	34º 33 ' 23"S 138º 41' 35"E		
So0496	9 Jun 07	SW of S4 RAC Roadside no ALS sprayed soursob present	SA	34º 32 ' 50"S 138º 41' 17"E		
So0497	9 Jun 07	SW of S4 RAC Roadside no ALS sprayed soursob present	SA	34º 32 ' 47"S 138º 41' 14"E		
So0498	9 Jun 07	W of S4 RAC Roadside no ALS sprayed soursob present	SA	34º 32 ' 43"S 138º 41' 14"E		
So0499	9 Jun 07	W of S4 RAC Roadside no ALS sprayed soursob present	SA	34º 32 ' 47"S 138º 41' 14"E		
So0500	9 Jun 07	W of S4 RAC Roadside no ALSsprayd soursob present Lg Hd	SA	34º 32 ' 46"S 138º 41' 14"E		
So0501	9 Jun 07	W of S4 RAC Roadside	SA	34º 32 ' 41"S 138º 41' 08"E		
So0502	9 Jun 07	NW of S4 RAC Roadside	SA	34º 32 ' 19"S 138º 40' 24"E		
So0503	9 Jun 07	NW of S4 RAC Roadside ALS sprayed looks resistant	SA	34º 32 ' 13"S 138º 40' 11"E	P31	
So0504	9 Jun 07	NW of S4 RAC Roadside Looks like res patch maybe dying	SA	34º 32 ' 14"S 138º 40' 12"E	P31	
So0505	9 Jun 07	N of S4 RAC Roadside by Dump looks res sprayed patch	SA	34º 32 ' 04"S 138º 340 32"E	P31	
So0506	15 Aug 07	Western Australian 092 Kathryn McCarren Myalup	WA	33º 06.595 ' S 115º 42.473' E	Res P25	
So0507	15 Aug 07	Western Australian 094A Kathryn McCarren Bussell highway	WA	33º 39.411 ' S 115º 24.709' E		
So0508	15 Aug 07	Western Australian 094A Kathryn McCarren Bussell highway	WA	33º 39.411 ' S 115º 24.709' E	Sus P25	
So0509	15 Aug 07	Western Australian 094B Kathryn McCarren Bussell highway	WA	33º 39.411 ' S 115º 24.709' E	Res P35	
So0509CT33B7	3 Aug 08	Regen From So509 23July 2008	WA	33º 39.411 ' S 115º 24.709' E	Res CT33	
So0510	15 Aug 07	Western Australian 094B Kathryn McCarren Bussell highway	WA	33º 39.411 ' S 115º 24.709' E		
So0511	15 Aug 07	Western Australian 094C Kathryn McCarren Bussell highway	WA	33º 39.411 ' S 115º 24.709' E		
So0512	15 Aug 07	Western Australian 094C Kathryn McCarren Bussell highway	WA	33º 39.411 ' S 115º 24.709' E		
So0513	15 Aug 07	Western Australian 094C Kathryn McCarren Bussell highway	WA	33º 39.411 ' S 115º 24.709' E		
So0514	15 Aug 07	Western Australian 095 Kathryn McCarren Fonto cheese	WA	33º 48.441 ' S 115º 07.293' E		
So0515	15 Aug 07	Western Australian 095 Kathryn McCarren Fonto cheese	WA	33º 48.441 ' S 115º 07.293' E		
So0516	15 Aug 07	Western Australian 095 Kathryn McCarren Fonto cheese	WA	33º 48.441 ' S 115º 07.293' E	Sus P25	
So0517	15 Aug 07	Western Australian 96A Kathryn McCarren Witchcliffe	WA	34º 01.354 ' S 115º 05.948 ' E		
So0518	15 Aug 07	Western Australian 96A Kathryn McCarren Witchcliffe	WA	34º 01.354 ' S 115º 05.948 ' E		
So0519	15 Aug 07	Western Australian 96A Kathryn McCarren Witchcliffe	WA	34º 01.354 ' S 115º 05.948 ' E		
So0520	15 Aug 07	Western Australian 96A Kathryn McCarren Witchcliffe	WA	34º 01.354 ' S 115º 05.948 ' E		
So0521	15 Aug 07	Western Australian 96A Kathryn McCarren Witchcliffe	WA	34º 01.354 ' S 115º 05.948 ' E		
So0522	15 Aug 07	Western Australian 96A Kathryn McCarren Witchcliffe	WA	34º 01.354 ' S 115º 05.948 ' E		
So0523	15 Aug 07	Western Australian 96B Kathryn McCarren Witchcliffe	WA	34º 01.354 ' S 115º 05.948 ' E	Sus P25 P35	

So0524	15 Aug 07	Western Australian 96B Kathryn McCarren Witchcliffe	WA	34º 01.354 ' S 115º 05.948 ' E	Sus P35	
So0525	15 Aug 07	Western Australian 98 Kathryn McCarren Augusta No Seed	WA	34º 27.052 ' S 115º 08.861 ' E	P31	
So0526	16 Aug 07	Western Australian 102 Kathryn McCarren Farm edge roadside	WA	34º 26.664 ' S 115º 54.054 ' E		
So0527	16 Aug 07	Western Australian 102 Kathryn McCarren Farm edge roadside	WA	34º 26.664 ' S 115º 54.054 ' E	Sus P25	
So0528	16 Aug 07	Western Australian 102 Kathryn McCarren Farm edge roadside	WA	34º 26.664 ' S 115º 54.054 ' E		
So0529	17 Aug 07	Western Australian 110 Kathryn McCarren Nanurup	WA	34º 59.628 ' S 118º 03.800 ' E	Sus P25	
So0530	17 Aug 07	Western Australian 111 Kathryn McCarren Porangerup	WA	34º 39.400 ' S 117º 53.356 ' E	Sus P25 P35	
So0531	17 Aug 07	Western Australian 112 Kathryn McCarren Mt Barker	WA	34º 38.048 ' S 117º 39.984 ' E		
So0532	17 Aug 07	Western Australian 112 Kathryn McCarren Mt Barker	WA	34º 38.048 ' S 117º 39.984 ' E	Sus P35	P25R?
So0533	17 Aug 07	Western Australian 112 Kathryn McCarren Mt Barker	WA	34º 38.048 ' S 117º 39.984 ' E		
So0534	17 Aug 07	Western Australian 115A Kathryn McCarren Aurther River	WA	33º 20.133 ' S 117º 02.006 ' E	Sus P25	
So0535	17 Aug 07	Western Australian 115A Kathryn McCarren Aurther River	WA	33º 20.133 ' S 117º 02.006 ' E		
So0536	17 Aug 07	Western Australian 115A Kathryn McCarren Aurther River	WA	33º 20.133 ' S 117º 02.006 ' E		
So0537	17 Aug 07	Western Australian 115A Kathryn McCarren Aurther River	WA	33º 20.133 ' S 117º 02.006 ' E		
So0538	17 Aug 07	Western Australian 115A Kathryn McCarren Aurther River	WA	33º 20.133 ' S 117º 02.006 ' E		
So0539	17 Aug 07	Western Australian 115B Kathryn McCarren Aurther River	WA	33º 20.133 ' S 117º 02.006 ' E		
So0540	17 Aug 07	Western Australian 115B Kathryn McCarren Aurther River	WA	33º 20.133' S 117º 02.006 ' E		
So0541	17 Aug 07	Western Australian 117 Kathryn McCarren Bannister	WA	32º 40.754 ' S 116º 31.169 ' E	Sus P25	
So0542	17 Aug 07	Western Australian 117 Kathryn McCarren Bannister	WA	32º 40.754 ' S 116º 31.169 ' E		
So0543	17 Aug 07	Western Australian 117 Kathryn McCarren Bannister	WA	32º 40.754 ' S 116º 31.169 ' E		
So0544	17 Aug 07	Western Australian 117 Kathryn McCarren Bannister	WA	32º 40.754 ' S 116º 31.169 ' E		
So0545	17 Aug 07	Western Australian 118 Kathryn McCarren	WA	32º 24.618 ' S 116º 16.880 ' E		
So0546	17 Aug 07	Western Australian 118 Kathryn McCarren	WA	32º 24.618 ' S 116º 16.880 ' E		
So0547	17 Aug 07	Western Australian 118 Kathryn McCarren	WA	32º 24.618 ' S 116º 16.880 ' E		
So0548	17 Aug 07	Western Australian 118 Kathryn McCarren **No Seed**	WA	32º 24.618 ' S 116º 16.880 ' E		
So0549	07 Sep 07	Holden Hill SA Corner Tarton and Lyons Roads	SA	34º 51 ' 25.92"S 138º 39' 57.79"E	Res 8/30 16A	LgePl
So0550	07 Sep 07	Holden Hill SA Corner Tarton and Lyons Roads	SA	34º 51 ' 26.00"S 138º 39' 57.79"E	Res 30/3016A	LgePl
So0551	07 Sep 07	Holden Hill SA Corner Tarton and Lyons Roads	SA	34º 51 ' 26.18"S 138º 39' 57.79"E	Res 20/4016A	LgePl
So0552	07 Sep 07	Holden Hill SA Corner Tarton and Lyons Roads	SA	34º 51 ' 26.31"S 138º 39' 57.79"E	Res 8/40 16A	P31 LP
So0553	07 Sep 07	Holden Hill SA Corner Tarton and Lyons Roads	SA	34º 51 ' 26.40"S 138º 39' 57.79"E	P31	
So0554	10 Oct 07	Toowong Brisbane By road and buildings	Qu	27º 28 ' 34.00"S 153º 00' 07.17"E	P31	
So0555	13 Nov 07	Adelaide 31 Russel St in house back yard NW cnr large plant	SA	34º 55 ' 57.52"S 138º 35' 47.11"E	P31 P35	
So0556	5 Nov 07	Globe Derby Paddock Green Fields #1	SA	34º 47 ' 36.40"S 38º 35' 38.52"E	P35 Sus	
So0557	5 Nov 07	Globe Derby Paddock Green Fields #2	SA	34º 47 ' 36.40"S 38º 35' 38.52"E	P35 Sus	
So0558	5 Nov 07	Globe Derby Paddock Green Fields #3	SA	34º 47 ' 36.40"S 38º 35' 38.52"E		

So0559	5 Nov 07	Globe Derby Paddock Green Fields #4	SA	34º 47 ' 36.40"S 38º 35' 38.52"E		
So0560	5 Nov 07	Globe Derby Paddock Green Fields #5	SA	34º 47 ' 36.40"S 38º 35' 38.52"E		
So0561	27 Nov 07	1A Roseworthy S4 Roadside East edge S4	SA	34º 32 ' 59.32"S 138º 41' 32.87"E	P32 Sus	
So0562	27 Nov 07	2A Roseworthy S4 Roadside East edge S4	SA	34º 32 ' 59.28"S 138º 41' 32.87"E	P32 Sus	
So0563	27 Nov 07	3A Roseworthy S4 Roadside East edge S4	SA	34º 32 ' 59.24"S 138º 41' 32.87"E	P32 .5Res .5Sus	
So0564	27 Nov 07	4A Roseworthy S4 Roadside East edge S4	SA	34º 32 ' 59.20"S 138º 41' 32.87"E	P32 Res	
So0565	27 Nov 07	5A Roseworthy S4 Roadside East edge S4	SA	34º 32 ' 59.16"S 138º 41' 32.87"E	P32 Sus	
So0566	27 Nov 07	6B Roseworthy S4 Roadside East edge S4	SA	34º 32 ' 55.96"S 138º 41' 32.45"E	P32 Res	
So0567	27 Nov 07	7B Roseworthy S4 Roadside East edge S4	SA	34º 32 ' 55.92"S 138º 41' 32.45"E	P32 Res	
So0568	27 Nov 07	8B Roseworthy S4 Roadside East edge S4	SA	34º 32 ' 55.88"S 138º 41' 32.45"E	P32 Res	
So0569	27 Nov 07	9B Roseworthy S4 Roadside East edge S4	SA	34º 32 ' 55.84"S 138º 41' 32.45"E	P32 Res	
So0570	27 Nov 07	10B Roseworthy S4 Roadside East edge S4	SA	34º 32 ' 55.80"S 138º 41' 32.45"E	P32 Sus	
So0571	25 Apr 08	Balaklava	SA	34º 08 ' 42.72"S 138º 25' 09.01"E	P35 Sus	
So0572	27 Sep 08	Wasleys Yellow Flower	SA	34º 28 ' 21.00"S 138º 41' 11.00"E		
So0573	27 Sep 08	Wasleys Whitish Yellow Flower	SA	34º 28 ' 21.00"S 138º 41' 11.00"E		
So0574	3 Nov 08	GeneFlowB By 16 in Waite Lab Field	SA	28º 25 ' 93.00"S 149º 42' 05.00"E		
So0575						
So0576						
So0577						
So0578						
So0579						
So0580						

Appendix 2. GENE FLOW DATA

Table 18 or S1. Number of capitulum seed heads harvested from an individual plant of susceptible *S. oleraceus* line So115 grown within 100mm of an individual resistant *S. oleraceus* line So057 over a six week period. The table also displays the number of seed progeny from So115 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

Susceptible *S. oleraceus* line and head harvest # [a]		# of capitulum heads harvested	Seed # sown	Seedling # treated [b]	Result 30 days after treatment # Dead **Susceptible**	# Alive **Resistant**
S01So115	H033	1	80	38	38	0
S01So115	H037	2	100	76	76	0
S01So115	H079	1	0	0	0	0
S01So115	H091	2	30	24	24	0
S01So115	H136	2	140	102	102	0
S01So115	H168	3	160	31	31	0
S01So115	H189	4	100	6	6	0
S01So115	H207	6	200	6	6	0
S01So115	H222	7	100	17	17	0
S01So115	H235	4	100	28	28	0
S01So115	H251	3	30	1	1	0
Totals		35	1040	329	329	0

[a] Capitulum heads harvested as mature from mid February 2007 to late March 2007.
[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

Table 18B or R1. Number of capitulum seed heads harvested from an individual plant of resistant *S. oleraceus* line So057 grown within 100mm of an individual susceptible *S. oleraceus* line So115 over a six week period. The table also displays the number of seed progeny from selected capitulum heads of So057 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

Resistant *S. oleraceus* line and head harvest # [a]		# of capitulum heads harvested	Seed # sown	Seedling # treated [b]	Result 30 days after treatment # Dead **Susceptible**	# Alive **Resistant**
R01So057	H064	1				
R01So057	H080	2				
R01So057	H131	2				
R01So057	H138	2				
R01So057	H180	1	50	38	1	37
R01So057	H207	2				
R01So057	H233	2	20	0	0	0
R01So057	H244	3	20	4	0	4
R01So057	H252	1				
Totals		16	90	42	1	41

[a] Capitulum heads harvested as mature from mid February 2007 to late March 2007.
[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

Table S2. Number of capitulum seed heads harvested from an individual plant of susceptible *S. oleraceus* line So115 grown within 100mm of an individual resistant *S. oleraceus* line So101 over a six week period. The table also displays the number of seed progeny from So115 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

					Result 30 days after treatment	
Susceptible *S. oleraceus* line and head harvest # [a]		# of capitulum heads harvested	Seed # sown	Seedling # treated [b]	# Dead **Susceptible**	# Alive **Resistant**
S02So115	H003	1	50	6	6	0
S02So115	H015	1	50	10	8	2 [S1]
S02So115	H032	2	150	18	1	17 [S2 & S3]
S02So115	H049	1	100	44	44	0
S02So115	H063	1	100	16	16	0
S02So115	H078	4	200	80	80	0
S02So115	H090	1	80	55	55	0
S02So115	H109	1	18	3	3	0
S02So115	H115	4	150	23	23	0
S02So115	H135	3	80	22	20	2
S02So115	H146	3	120	44	41	3
S02So115	H152	3	120	56	56	0
S02So115	H188	4	140	8	8	0
S02So115	H206	5	200	10	10	0
S02So115	H221	3	120	12	12	0
Totals		37	1678	407	383	24

[a] Capitulum heads harvested as mature from mid February 2007 to late March 2007.

[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

Table R2. Number of capitulum seed heads harvested from an individual plant of resistant *S. oleraceus* line So101 grown within 100mm of an individual susceptible *S .oleraceus* line So115 over a six week period. The table also displays the number of seed progeny from selected capitulum heads of So101 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

					Result 30 days after treatment	
Resistant *S. oleraceus* line and head harvest # [a]		# of capitulum heads harvested	Seed # sown	Seedling # treated [b]	# Dead **Susceptible**	# Alive **Resistant**
R02So101	H079	2	150	29	4	25
R02So101	H122	1	150	44	0	44
R02So101	H173	3	200	8	0	8
R02So101	H179	1	100	0	0	0
Totals		7	600	81	4	77

[a] Capitulum heads harvested as mature from mid February 2007 to late March 2007.

[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

Table S3. Number of capitulum seed heads harvested from an individual plant of susceptible *S. oleraceus* line So115 grown within 100mm of an individual resistant *S. oleraceus* line So102 over a six week period. The table also displays the number of seed progeny from So115 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

				Result 30 days after treatment	
Susceptible *S. oleraceus* line and head harvest # [a]	# of capitulum heads harvested	Seed # sown	Seedling # treated [b]	# Dead **Susceptible**	# Alive **Resistant**
S03So115 H134	1	60	60	0	60
S03So115 H151	2	150	70	0	70
S03So115 H167	2	100	18	18	0
S03So115 H187	2	60	1	1	0
S03So115 H205	2	120	27	27	0
S03So115 H245	2	80	15	0	15
Totals	11	570	191	46	145

[a] Capitulum heads harvested as mature from mid February 2007 to late March 2007.

[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

Table R3. Number of capitulum seed heads harvested from an individual plant of resistant *S. oleraceus* line So102 grown within 100mm of an individual susceptible *S .oleraceus* line So115 over a six week period. The table also displays the number of seed progeny from selected capitulum heads of So102 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

				Result 30 days after treatment	
Resistant *S. oleraceus* line and head harvest # [a]	# of capitulum heads harvested	Seed # sown	Seedling # treated [b]	# Dead **Susceptible**	# Alive **Resistant**
R03So102 H165	3	150	140	0	140
Totals	3	150	140	0	140

[a] Capitulum heads harvested as mature from mid February 2007 to late March 2007.

[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

Table S4. Number of capitulum seed heads harvested from an individual plant of susceptible *S. oleraceus* line So115 grown within 100mm of an individual resistant *S. oleraceus* line So106 over a six week period. The table also displays the number of seed progeny from So115 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

					Result 30 days after treatment	
Susceptible *S. oleraceus* line and head harvest # [a]		# of capitulum heads harvested	Seed # sown	Seedling # treated [b]	# Dead **Susceptible**	# Alive **Resistant**
S04So115	H166	4	150	5	5	0
S04So115	H220	5	200	50	50	0
S04So115	H240	3	150	2	2	0
S04So115	H248	4	200	20	20	0
Totals		16	700	77	77	0

[a] Capitulum heads harvested as mature from mid February 2007 to late March 2007.
[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

Table R4. Number of capitulum seed heads harvested from an individual plant of resistant *S. oleraceus* line So106 grown within 100mm of an individual susceptible *S .oleraceus* line So115 over a six week period. The table also displays the number of seed progeny from selected capitulum heads of So106 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

					Result 30 days after treatment	
Resistant *S. oleraceus* line and head harvest # [a]		# of capitulum heads harvested	Seed # sown	Seedling # treated [b]	# Dead **Susceptible**	# Alive **Resistant**
R04So106	H231	5	200	76	0	76
Totals		5	200	76	0	76

[a] Capitulum heads harvested as mature from mid February 2007 to late March 2007.
[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

Table S5. Number of capitulum seed heads harvested from an individual plant of susceptible *S. oleraceus* line So115 grown within 100mm of an individual resistant *S. oleraceus* line So108 over a six week period. The table also displays the number of seed progeny from So115 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

					Result 30 days after treatment	
Susceptible *S. oleraceus* line and head harvest # [a]		# of capitulum heads harvested	Seed # sown	Seedling # treated [b]	# Dead **Susceptible**	# Alive **Resistant**
S05So115	H150	2	120	60	50	10 [S4]
S05So115	H185	8	600	120	159	1 [S5]
S05So115	H204	3	200	30	30	0
S05So115	H219	7	500	160	160	0
S05So115	H237	7	400	150	150	0
S05So115	H249	6	300	80	80	0
Totals		33	2120	600	629	11

[a] Capitulum heads harvested as mature from mid February 2007 to late March 2007.

[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

Table R5. Number of capitulum seed heads harvested from an individual plant of resistant *S. oleraceus* line So108 grown within 100mm of an individual susceptible *S .oleraceus* line So115 over a six week period. The table also displays the number of seed progeny from selected capitulum heads of So108 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

					Result 30 days after treatment	
Resistant *S. oleraceus* line and head harvest # [a]		# of capitulum heads harvested	Seed # sown	Seedling # treated [b]	# Dead **Susceptible**	# Alive **Resistant**
R05So108	H220	4	30	20	0	20
R05So108	H242	10	50	45	0	45
Totals		14	80	65	0	65

[a] Capitulum heads harvested as mature from mid February 2007 to late March 2007.

[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

Table S6. Number of capitulum seed heads harvested from an individual plant of susceptible *S. oleraceus* line So115 grown within 100mm of an individual resistant *S. oleraceus* line So125 over a six week period. The table also displays the number of seed progeny from So115 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

					Result 30 days after treatment	
Susceptible *S. oleraceus* line and head harvest # [a]		# of capitulum heads harvested	Seed # sown	Seedling # treated [b]	# Dead **Susceptible**	# Alive **Resistant**
S06So115	H004	1	200	1	0	1
S06So115	H031	5	200	120	120	0
S06So115	H048	1	75	15	15	0
S06So115	H062	1	100	0	0	0
S06So115	H077	2	125	15	15	0
S06So115	H088	2	50	15	15	0
S06So115	H113	1	50	28	27	1
S06So115	H132	3	100	56	56	0
S06So115	H145	4	135	38	0	38
S06So115	H149	3	150	0	0	0
S06So115	H165	5	200	62	62	0
S06So115	H184	6	350	95	70	25 [S6&S7]
S06So115	H203	4	300	16	16	0
S06So115	H218	6	300	39	39	0
S06So115	H233	3	150	30	30	0
Totals		47	2485	530	465	65

[a] Capitulum heads harvested as mature from mid February 2007 to late March 2007.

[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

Table R6. Number of capitulum seed heads harvested from an individual plant of resistant *S. oleraceus* line So125 grown within 100mm of an individual susceptible *S .oleraceus* line So115 over a six week period. The table also displays the number of seed progeny from selected capitulum heads of So125 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

					Result 30 days after treatment	
Resistant *S. oleraceus* line and head harvest # [a]		# of capitulum heads harvested	Seed # sown	Seedling # treated [b]	# Dead **Susceptible**	# Alive **Resistant**
R06So125	H030	3	100	1	0	1
R06So125	H038	1	60	50	0	50
R06So125	H110	2	80	0	0	0
R06So125	H126	1	120	1	0	1
R06So125	H219	2	100	23	0	23
R06So125	H229	4	200	20	0	20
Totals		13	660	95	0	95

[a] Capitulum heads harvested as mature from mid February 2007 to late March 2007.

[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

Table S7. Number of capitulum seed heads harvested from an individual plant of susceptible *S. oleraceus* line So115 grown within 100mm of an individual resistant *S. oleraceus* line So145 over a six week period. The table also displays the number of seed progeny from So115 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

					Result 30 days after treatment	
Susceptible *S. oleraceus* line and head harvest # [a]		# of capitulum heads harvested	Seed # sown	Seedling # treated [b]	# Dead **Susceptible**	# Alive **Resistant**
S07So115	H020	1	60	20	19	1
S07So115	H047	3	300	140	139	1 [S8]
S07So115	H061	2	240	34	34	0
S07So115	H097	1	85	15	15	0
S07So115	H111	1	100	40	38	2 [S9]
S07So115	H112	2	100	6	0	6
S07So115	H131	5	160	40	35	5 [S10]
S07So115	H143	3	125	60	57	3
S07So115	H182	2	120	2	2	0
S07So115	H183	6	250	70	70	0
S07So115	H217	5	200	80	79	1 [S11]
S07So115	H232	4	200	70	70	0
S07So115	H246	5	200	80	80	0
Totals		40	2140	657	638	19

[a] Capitulum heads harvested as mature from mid February 2007 to late March 2007.

[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

Table R7. Number of capitulum seed heads harvested from an individual plant of resistant *S. oleraceus* line So145 grown within 100mm of an individual susceptible *S .oleraceus* line So115 over a six week period. The table also displays the number of seed progeny from selected capitulum heads of So145 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

					Result 30 days after treatment	
Resistant *S. oleraceus* line and head harvest # [a]		# of capitulum heads harvested	Seed # sown	Seedling # treated [b]	# Dead **Susceptible**	# Alive **Resistant**
R07So145	H093	5	175	60	0	60
R07So145	H175	4	170	38	0	38
R07So145	H251	5	150	28	0	28
Totals		14	495	126	0	126

[a] Capitulum heads harvested as mature from mid February 2007 to late March 2007.

[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

Table S8. Number of capitulum seed heads harvested from an individual plant of susceptible *S. oleraceus* line So115 grown within 100mm of an individual resistant *S. oleraceus* line So149 over a six week period. The table also displays the number of seed progeny from So115 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

				Result 30 days after treatment	
Susceptible *S. oleraceus* line and head harvest # [a]	# of capitulum heads harvested	Seed # sown	Seedling # treated [b]	# Dead **Susceptible**	# Alive **Resistant**
S08So115 H130	2	50	16	16	0
S08So115 H164	3	250	26	26	0
S08So115 H181	4	350	1	1	0
S08So115 H202	4	250	12	12	0
S08So115 H216	5	350	35	35	0
S08So115 H239	4	150	1	1	0
S08So115 H247	1	40	0	0	0
Totals	23	1440	91	91	0

[a] Capitulum heads harvested as mature from mid February 2007 to late March 2007.

[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

Table R8. Number of capitulum seed heads harvested from an individual plant of resistant *S. oleraceus* line So149 grown within 100mm of an individual susceptible *S .oleraceus* line So115 over a six week period. The table also displays the number of seed progeny from selected capitulum heads of So149 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

				Result 30 days after treatment	
Resistant *S. oleraceus* line and head harvest # [a]	# of capitulum heads harvested	Seed # sown	Seedling # treated [b]	# Dead **Susceptible**	# Alive **Resistant**
R08So149 H228	5	160	64	0	64
Totals	5	160	64	0	64

[a] Capitulum heads harvested as mature from mid February 2007 to late March 2007.

[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

Table S9. Number of capitulum seed heads harvested from an individual plant of susceptible *S. oleraceus* line So115 grown within 100mm of an individual resistant *S. oleraceus* line So154 over a six week period. The table also displays the number of seed progeny from So115 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

					Result 30 days after treatment	
Susceptible *S. oleraceus* line and head harvest # [a]		# of capitulum heads harvested	Seed # sown	Seedling # treated [b]	# Dead **Susceptible**	# Alive **Resistant**
S09So115	H014	1	150	56	56	0
S09So115	H046	1	150	102	101	1
S09So115	H060	1	150	80	76	4
S09So115	H144	2	100	20	20	0
S09So115	H163	1	100	11	11	0
S09So115	H200	3	100	28	28	0
S09So115	H215	2	150	85	82	3
Totals		11	900	382	374	8

[a] Capitulum heads harvested as mature from mid February 2007 to late March 2007.

[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

Table R9. Number of capitulum seed heads harvested from an individual plant of resistant *S. oleraceus* line So154 grown within 100mm of an individual susceptible *S .oleraceus* line So115 over a six week period. The table also displays the number of seed progeny from selected capitulum heads of So154 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

					Result 30 days after treatment	
Resistant *S. oleraceus* line and head harvest # [a]		# of capitulum heads harvested	Seed # sown	Seedling # treated [b]	# Dead **Susceptible**	# Alive **Resistant**
R09So154	H014	2	60	27	2	25
R09So154	H015	1	50	0	0	0
R09So154	H170	1	100	44	0	44
Totals		4	210	71	2	69

[a] Capitulum heads harvested as mature from mid February 2007 to late March 2007.

[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

Table S10. Number of capitulum seed heads harvested from an individual plant of susceptible *S. oleraceus* line So115 grown within 100mm of an individual resistant *S. oleraceus* line So155 over a six week period. The table also displays the number of seed progeny from So115 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

					Result 30 days after treatment	
Susceptible *S. oleraceus* line and head harvest # [a]		# of capitulum heads harvested	Seed # sown	Seedling # treated [b]	# Dead **Susceptible**	# Alive **Resistant**
S10So115	H006	1	100	75	72	3
S10So115	H017	1	80	60	60	0
S10So115	H045	3	180	130	125	5
S10So115	H059	1	80	30	30	0
S10So115	H087	1	10	5	5	0
S10So115	H096	1	30	20	20	0
S10So115	H107	1	5	1	1	0
S10So115	H110	3	10	20	20	0
S10So115	H129	1	20	10	8	2
S10So115	H142	2	30	10	10	0
S10So115	H162	1	20	7	7	0
S10So115	H180	1	30	8	8	0
S10So115	H199	2	80	30	30	0
S10So115	H214	5	200	39	36	0
S10So115	H238	2	120	46	46	3
Totals		26	995	491	478	13

[a] Capitulum heads harvested as mature from mid February 2007 to late March 2007.
[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

Table R10. Number of capitulum seed heads harvested from an individual plant of resistant *S. oleraceus* line So155 grown within 100mm of an individual susceptible *S .oleraceus* line So115 over a six week period. The table also displays the number of seed progeny from selected capitulum heads of So155 sown, the number of 2 leaf emerged seedlings treated with chlorsulfuron 15g ai ha^{-1} and the number of plants that were either dead or alive 30 days after treatment.

					Result 30 days after treatment	
Resistant *S. oleraceus* line and head harvest # [a]		# of capitulum heads harvested	Seed # sown	Seedling # treated [b]	# Dead **Susceptible**	# Alive **Resistant**
R10So155	H030	3	100	115	0	115
R10So155	H038	1	40	21	0	21
Totals		4	140	136	0	136

[a] Capitulum heads harvested as mature from mid February 2007 to late March 2007.
[b] Number of established 2 leaf seedlings treated with chlorsulfuron.

Appendix 3. SOURCE OF CHEMICALS

Biochemical	**Source**
Taq DNA Polymerase in storage buffer A	Promega (Australia)
2-mercaptoethanol	Riedel-deHaën (Germany)
Agar Bacteriological (Agar No.1)	Oxoid (England)
Agarose 1	Amresco (USA)
Chloroform	BDH (Australia)
CTAB	Merck (Germany)
EDTA	BDH (Australia)
EtBr	Amresco (USA)
Ethanol	BDH (Australia)
Isoamyl alcohol	UNIVAR (Australia)
$MgCl_2$	Promega (Australia)
NaCl	BDH (Australia)
Tris	Amresco (USA)
rATP	Promega (Australia)
Tru9I Restriction enzyme	Promega (Australia)
T4 DNA ligase	Promega (Australia)
PCR Nucleotide mix	Promega (Australia)
Platinum Taq High Fidelity	Invitrogen (Australia)
Mse1	New England Biolabs (Australia)
PstI cutting enzyme	Promega (Australia)
dNTP's	Promega(Australia)
10X buffer Mg free	Promega (Australia)
6X Loading Dye	Promega (Australia)
DNeasy plant minikit DNA extraction	Qiagen (Australia)

Appendix 4. SOURCE OF EQUIPMENT

96 well plate	Adelab (Australia)
Sealex 96 well plate adhesive film	Adelab (Australia)
Resch mixing mill	MEP (Australia)
Bonson container	Mick Savill (Australia)
1.5mL centrifuge tubes	Adelab (Australia)
Seed envelopes 90X140mm	Spicer Tudor (Australia)

Appendix 5. OGLIONUCLEOTIDES

All oglionucleotide primers and their application are shown in the table below

Sequencing Primers		
M*se*1 adapter	M-adap1	5’ – GAC GAT GAG TCC TGA G – 3’
M*se*1 adapter	M-adap2	5’ – TAC TCA GGA CTC AT – 3’
P*st*1 adapter	P-adap1	5’ – CTC GTA GAC TGC GTA CAT GCA – 3’
P*st*1 adapter	P-adap2	5’ – TGT ACG CAG TCT AC – 3’

Appendix 5B. OGLIGONUCLEOTIDES

Annealing of M*se*I adapters

ADAPTER 1

Supplier is Geneworks

M*se*I Adapter 1 (16 mer)

MW=4946.3

387.0 µg

TM=46.0^{o}C

78.2 n mol

5’ GAC GAT GAG TCC TGA G

Concentration of 200µM

ADAPTER 2

Supplier is Geneworks

M*se*I Adapter 2 (14 mer)

MW=4222.8

189.5 µg

TM=40.0^{o}C

44.9 n mol

5’ TAC TCA GGA CTC AT

Concentration of 200µM

PROCEDURE

Add 50µL of Adapter 1 and 50µL of Adapter 2 to a PCR tube.

Add 100µL of MQ water (nanopure sterile water) to the tube.

This gives 200µL at a concentration of 5µM.

In a thermocycler machine heat to 90°C for 3 minutes then remove from thermocycler and allow to settle at room temperature for 30 minutes.

Store at -20°C.

Appendix 5C. OGLIGONUCLEOTIDES

Annealing of P*st*I adapters

ADAPTER 1

Supplier is Geneworks

P*st*I Adapter 1 (21 mer)

MW=6406.2

494.3 µg

TM=54.0°C

77.2 n mol

5’ CTC GTA GAC TGC GTA CAT GCA

Concentration of 200µM

ADAPTER 2

Supplier is Geneworks

P*st*I Adapter 2 (14 mer)

MW=4238.8

229.3 µg

TM=42.0°C

54.1 n mol

5’ TGT ACG CAG TCT AC

Concentration of 200µM

PROCEDURE

Add 5µL of Adapter 1 and 5µL of Adapter 2 to a PCR tube.

Add 190µL of MQ water (nanopure sterile water) to the tube.

This gives 200µL at a concentration of 5µM.

In a thermocycler machine heat to 90°C for 3 minutes then remove from thermocycler and allow to settle at room temperature for 30 minutes.

Store at -20°C.

Appendix 5D. OGLIGONUCLEOTIDES

M*se*1 + 1 primer C

Supplier is Geneworks

M*se*I C (17 mer)

MW=5234.5

195.1 µg

TM=44.0^{o}C

37.0 n mol

5’ - GAT GAG TCC TGA GTA AC- 3’

Concentration of 50µM

PROCEDURE

Dilute to 75ng/µL

10µL of 1µg/µL PstI A + 123µL of nanopure water.

Appendix 5E. OGLIGONUCLEOTIDES

P*st*1 + 1 primer A

Supplier is Geneworks

PstI A (17 mer)

MW=5219.4

320.2 µg

TM=47.0^{o}C

61.4 n mol

5' – GAC TGC GTA CAT GCA GA- 3'

Concentration of 50µM

PROCEDURE

Dilute to 75ng/µL

10µL of 1µg/µL PstI A + 123µL of nanopure water.

Appendix 5F. OGLIGONUCLEOTIDES

M*se*1 CC

Supplier is Geneworks

Mse1 CC (17 mer)

MW=5210.4

273.9 μg

TM=47.0^{o}C

56.6 n mol

5’ – GAT GAG TCC TGA GTA CC- 3’

Concentration of 1μg/μL ie 1000ng/μL

PROCEDURE

10μL of 1μg/μL M*se*1 CC + 390μL of nanopure water.=400μL of 25ng/μL

Appendix 5G. OGLIGONUCLEOTIDES

P*st*1 AC Fluorescent hex green

Supplier is Geneworks

P*st*1 AC (17 mer)

MW=6253.8

101.9 μg

TM=50.0°C

16.3 n mol

5' – 7GA CTG CGT ACA TGC AGA C- 3'

Concentration of 1μg/μL ie 1000ng/μL

PROCEDURE

5μL of 1μg/μL P*st*1 AC + 195μL of nanopure water.=200μL of 25ng/μL

Appendix 5H. OGLIGONUCLEOTIDES

M*se*1 CT

Supplier is Geneworks

Mse1 CC (18 mer)

MW=5538.7

240.4 μg

TM=46.0^{o}C

43.3 n mol

5' – GAT GAG TCC TGA GTA ACT- 3'

Concentration of 1μg/μL ie 1000ng/μL

PROCEDURE

10μL of 1μg/μL M*se*1 CC + 390μL of nanopure water.=400μL of 25ng/μL

Appendix 5I. OGLIGONUCLEOTIDES

P*st*1 AG Fluorescent hex green

Supplier is Geneworks

P*st*1 AG (18 mer)

MW=6225.0

83.8 µg

TM=50.0°C

13.5 n mol

5' – 5GA CTG CGT ACA TGC AGA G- 3'

Concentration of 1µg/µL ie 1000ng/µL

PROCEDURE

10µL of 1µg/µL M*se*1 CC + 390µL of nanopure water.=400µL of 25ng/µL

Appendix 5J. OGLIGONUCLEOTIDES

1.25mM dNTP's PCR nucleotide mix

Supplier is Bioline (Australia)

Contents and concentration 500μLof 25mM

PROCEDURE

Dilute to 75ng/μL

5μL of 25mM + 95μL of nanopure water = 100μL of 1.25mM dNTP's

Appendix 6. GELS, BUFFERS and MEDIA

1. Agarose gel

1.0 -1.5g of agarose was dissolved by heating in a microwave oven in 100mL of TAE buffer to make 1-1.5%(w/v) agarose.

Ethidium bromide was added to 0.5μg/mL

2. Miscellaneous buffers

6X Loading Buffer:

1 X TAE: 40mM Tris-HCl, 20 mM sodium acetate, 2 mM EDTA, adjusted to pH 7.8 with glacial acetic acid