# The biology and ecology of *Salsola australis* R.Br. (Chenopodiaceae) in southwest Australian cropping systems

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# Certification

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree.

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# Publications arising from this thesis

The following conference paper was adapted from Chapter 5

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Chapter 3

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# Abstract

*Salsola australis* is an introduced weed of crop and pasture systems in the Western Australian broad acre cropping and pasture region (wheat-belt). This thesis investigated the classification, biology and ecology of the genus *Salsola* in southwest Australia, as well as modelling the effectiveness of possible weed control practices. Prior to this research, *S. tragus* was the only recognised species of the *Salsola* genus within Australia. However, genetic analysis revealed that four genetically distinct putative taxa of the genus *Salsola* were found in southwest Australia, none of which were *S. tragus*. The taxa that is the most prevalent agricultural weed was classified as *S. australis*, but the other three putative taxa could not be matched to recognised species. All four taxa were diploid ($2n = 18$), as opposed to tetraploid ($2n = 36$) *S. tragus*. Within the agricultural system of southwest Australia, *S. australis* plants established throughout the year, although the majority of seed production occurred in late summer and autumn. Total seed production (138-7734 seeds per plant) and seed viability (7.6-62.8%) of *S. australis* were lower than that reported for other agricultural weed species of the *Salsola* genus. Seed dispersal occurred when the senesced plants broke free of their root system to become mobile. Wind driven plants travelled and shed seed over distances of 1.6 to 1247.2 m. Movement of approximately half the plants was restricted to less than 100 m by entanglement with other *S. australis* plants within the stand. Some seed was retained on the senesced plants, but the germinability of this seed fell to less than 2% in the two month period following plant senescence (i.e. a decline of 79%). Once seed shed into the soil seed bank, anywhere from 32.3 to 80.7% of the viable seeds germinated in the year following seed production, with the rest remaining dormant or degrading. A model of the life cycle of *S. australis* based on the population ecology data indicated that the dormant seed bank had very little effect on annual seedling recruitment, but seed dispersal from neighbouring populations had a large impact on population growth rate. Therefore, the most successful weed control measures were those that restricted seed dispersal from neighbouring populations, or those that were applied to all populations in the region rather than to a single population. Weed control techniques applied to a single population, without reducing seed dispersal, could not reduce population size.

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plants ($P_V$), reproductive to senesced plants ($P_R$), probability of easily shed or retained seeds germinating in the year following seed production ($P_{ESG}$ and $P_{RG}$), probability of easily shed or retained seeds becoming dormant ($P_{ESD}$ and $P_{RD}$) and the probability of dormant seeds germinating ($P_{DG}$). The diamonds represent fecundity, i.e. total viable easily shed or retained seed produced per plant ($S_{ES}$ and $S_R$).

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# Chapter 1
# Introduction

## Background

*Salsola tragus sensu lato* is a widespread species both in Australia and worldwide. This species is most common in dry, disturbed, saline or alkaline regions (Rilke 1999a). It is so widespread that the tumbleweeds formed by this species have become ingrained in western culture, and in films a tumbleweed blowing across a scene is often used to indicate a deserted location. Originating in Europe and Asia, *S. tragus* L. has since spread to Australia, North America and Africa and currently thrives on every continent with the exception of Antarctica (Rilke 1999a). Considerable expenditure occurs each year in attempting to control this species, especially in the USA (Smith 2004). *Salsola tragus* (reclassified by this thesis as *S. australis* R.Br.) has been in Australia for over 200 years, and historical evidence indicates that it arrived prior to European invasion (Brown 1810; Smith et al. 1980).

*Salsola tragus* is a highly successful and versatile weed in Australia, invading both native vegetation and ruderal habitats (Beadle 1981; Eldridge et al. 2006; Mitchell and Wilcox 1988; Westbrooke and Florentine 2005). Within broad scale cropping systems it is a weed of the summer fallow period. Like any summer weed, it uses water and nutrients that would ordinarily be available to the following crop, thus reducing crop yield potential (Mussell and Stewart 2004; Osten et al. 2006; Schillinger and Young 2000). *Salsola tragus* also delays seeding by blocking machinery, causing further reductions in crop yields (Tennant 2000). Within pastures, the young plants may cause oxalate poisoning in stock, and the prickly, senesced plants injure livestock (Jacob et al. 1992; Mussell and Stewart 2004; Petheram and Kok 2003). The pollen of populations of *S. tragus sensu lato* in the USA and Kuwait are a human allergen (Al-Dowaisan et al. 2004; Arbes et al. 2005), although this has not been investigated in Australia. The mobile tumbleweeds have considerable nuisance value, damaging infrastructure such as fences, and posing fire and traffic hazards (Mussell and Stewart 2004; Smith 2004).

In Australia this weed is of economic concern because it is poorly managed in cropping systems and no management options are available in pasture systems (Mussell and

Stewart 2004). Poor management may result from insufficient knowledge on the classification and biology of this weed.

Classification of *S. tragus sensu lato* has a confusing history worldwide, and in Australia the classification of variants is still under debate (Australian Plant Name Index 2005; Rilke 1999a). A result of the taxonomic confusion surrounding this weed is that the origin and status of this weed (native or exotic) in Australia is likewise under debate (Bean 2007). Correct classification and determination of the origin of this species in Australia may have a significant impact on management, in terms of quarantine status and potential for biological control.

There is very little data on the biology and population ecology of *S. tragus sensu lato* in Australia, especially where present in agricultural systems. While a considerable amount of information on the population ecology of this species has been amassed in other countries (predominately the USA), this will be of limited use in the different climate and soil types in Australia. Highly invasive weed species such as *S. tragus* that exist in a broad range of habitats commonly exhibit variation in seed germination patterns, life cycle phenology (i.e. from annual to perennial) and adaptations to disturbance and resource fluctuations (Clements et al. 2004). It is likely that the biology of Australian *S. tragus* populations will vary from those found on other continents. This is further confirmed by the morphological diversity observed within Australian populations of *S. tragus*, indicating a level of genetic diversity not observed within populations of this species on the other continents where it is an invasive weed, i.e. North and South America and Africa (Rilke 1999a). A thorough knowledge of the biology and population ecology of weed species is necessary for the development of successful management strategies (Cousens 1995; Holst et al. 2007; Jordan 1992; Kropff et al. 1996). The lack of data on the biology of *S. tragus* in Australian agricultural systems is likely to hamper the implementation of control strategies.

**Objectives**

The major objectives of this thesis include:

- To investigate the genetic and cytological variation within populations of *S. tragus sensu lato*.

- To investigate the biology and ecology of *S. tragus*, with specific reference to seed production, seed dispersal, soil seed bank characteristics, seedling establishment and survivorship.
- To model the population ecology of *S. tragus* and assess the effectiveness of potential weed control options.

**The study**

The available literature on *S. tragus sensu lato* is reviewed in Chapter 2. This review highlights the prevalence of this weed in Australia and the taxonomic confusion within the genus *Salsola*. Research into the biology and ecology of *S. tragus sensu lato* populations as they occur in agricultural systems outside Australia is summarised. Further, the agricultural impact of this species in Australia and the management options that are employed against this weed are discussed. The genetic and cytological characteristics of *S. tragus* populations within the wheat-belt of Western Australia are investigated in Chapters 3 and 4. Data on the seed biology, viability, germination temperature requirements and germination cues of *S. tragus* populations within the wheat-belt are presented in Chapter 5 and seed dispersal in Chapter 6. The population ecology of this species is discussed in Chapter 7, with particular regard to seedling establishment, survivorship and fecundity of wheat-belt populations. The population ecology data is modelled to investigate possible management options for *S. tragus* in the Australian cropping system in Chapter 8. The conclusions and implications of this research are discussed in Chapter 9.

# Chapter 2

# *Salsola tragus sensu lato* in Australia and worldwide: A review

## Introduction

*Salsola tragus sensu lato* is widespread throughout Australia, as well as the rest of the world. Within Australia *S. tragus* has colonised every climatic region of the mainland and survives in a wide range of natural and disturbed environments (Mitchell and Wilcox 1988). In the Western Australian wheat-belt *S. tragus* is a weed of cropping and pasture systems (Mussell and Stewart 2004). In the more arid rangeland regions it serves as forage for livestock. In disturbed areas it plays a role in ecological restoration, as it is one of the few early successional species that volunteers on highly degraded, alkaline or saline sites such as mine sites. Very little is known about the classification of Australian variants of the *Salsola* genus, the biology and ecology of these plants, the extent of their impact on agricultural systems or suitable weed control techniques for Australian populations of *S. tragus*. This review aims to summarise research conducted on populations of *S. tragus* in Australia and overseas, in an attempt to identify the areas where knowledge of Australian *S. tragus* is deficient and highlight profitable areas for future research into the management of this weed in native and agricultural environments.

## *Salsola tragus*

### *Phylogeny*

The genus *Salsola* L. is placed in the Chenopodiaceae family, which consists of 110 genera that are mainly annuals or small shrubs found in arid to semi-arid, saline, disturbed natural and agricultural environments (Kadereit et al. 2003). The Chenopodiaceae tribes that are native to Australia were estimated to result from a minimum of nine independent colonisation events (Kadereit et al. 2005). *Salsola tragus* is found within subfamily Salsoloideae, tribe Salsoleae (Rilke 1999a). Salsoleae is likely the tribe of most recent origin in Australia (Kadereit et al. 2005). *Salsola tragus* is the only species of this tribe found in Australia (Western Australian Herbarium 2007).

### *Taxonomy*

The *Salsola* genus consists of approximately 100-150 species, divided into nine sections (Mosyakin 1996; Rilke 1999b). *Salsola tragus* belongs to the section *Kali* Dumort.,

which contains 13 species. They are all annuals, differentiated by the length of the spines on the leaves and morphology of the wings and tepal lobes on the flowers and fruit (Table 2.1) (Rilke 1999b).

Table 2.1: Species classified within *Salsola* L. section *Kali* Dumort. and their leaf spine length and flower morphology, two of the morphological traits used to differentiate species (Rilke 1999b).

| Section *Kali* Dumort. | Leaf spine length (mm) | Flower morphology |
|---|---|---|
| *S. collina* Pall. | 1-4 | No wings |
| *S. griffithii* (Bunge) Freitag & Khani | | 5 wings, tepal lobes erect |
| *S. ikonnikovii* Iljin | | 5 wings, tepal lobes erect |
| *S. jacquemontii* Moq. | | 5 wings, tepal lobes papery |
| *S. kali* L. | | 5 wings, tepal lobes spine-like |
| *S. komarovii* Iljin | | 5 wings, tepal lobes papery, or no wings |
| *S. monoptera* Bunge | | 1 wing, tepal lobes short |
| *S. paulsenii* Litv. | | 5 wings, tepal lobes spine-like |
| *S. tamamschjanae* Iljin | | 5 wings, tepal lobes papery |
| *S. tragus* L. | | 5 wings, tepal lobes papery, or no wings |
| *S. zaidamica* Iljin | | No wings, fruit appressed at centre |
| *S. rosacea* L. | 0.1-0.3 | 5 wings, tepal lobes short, fruit appressed at centre |
| *S. tamariscina* Pall. | | |

*Name*

The name *Salsola* is from the Latin salsus, meaning salty, in reference to the salt tolerance evident in many species of this genus. The origin of *tragus* is less clear. The plant may have been named after Hieronymus Tragus, the Greek name for Jerome Bock (1498-1554), who was one of the founders of German botany. This species was originally classified as *S. kali* in Australia. The word *kali* originated from an Arabic word meaning the calcined ashes of *Salsola* or *Salicornia* species (Smith 1960). *Salsola*

*tragus* has a very wide range of common names internationally and in Australia is usually referred to as roly poly, prickly saltwort, soft buckbush or tumbleweed (Wilson 1984).

*Salsola tragus* is found worldwide, and has a high degree of intra-specific variation. The species likely originated in Europe or Asia, but has since spread throughout this continent, as well as North and South America, Africa and Australia (Mosyakin 1996; Rilke 1999a). Within the group *Salsola* section *Kali* Dumort., several species have a high level of intra-specific variation, which is combined with a low level of inter-specific diversity (Rilke 1999a; 1999b). As a result, accurate classification has proven to be challenging. Classification of *S. kali* and *S. tragus* is especially difficult as both have spines the same length (1-4 mm) and both produce fruit with five wings, although *S. tragus* can also produce fruit with no wings. Given the ease with which hybrids arise between these species and the high level of intra-specific variation, Rilke (1999b) has concluded that this group of species is in an active state of evolution. As a result, the assignation of botanical names to various species within the *Salsola* genus has a confusing history (Rilke 1999b; Young 1991).

*Salsola tragus* has previously been classified as *S. tragus* subsp. *iberica* Sennen and Pau, *S. iberica* (Sennen and Pau) Botschantzev ex Czerepanov, *S. australis* R. Brown, *S. kali* L. var. *tenuifolia* Tausch ex Moquin-Tandon, *S. kali* L. var. *angustifolia* Fenzl, *S. kali* L. var. *leptophylla* Bentham, *S. kali* L. var. *pseudotragus* G. Beck, *S. kali* L. var. *austroafricana* P. Allen, *S. pestifer* A. Nelsen and *S. ruthenica* Iljin (Mosyakin 1996; 2003; Rilke 1999a). *Salsola kali*, *S. tragus* and *S. australis* have been used almost interchangeably in the literature until recently and the identification of many other species has been inaccurate (Beatley 1973; Mosyakin 1996; 2003; Rilke 1999a; 1999b; Young 1991). In Australia, populations of *Salsola* were originally classified as *S. australis* and *S. macrophylla* R.Br. (Brown 1810). In Flora of Australia, Australian material was reclassified as *S. kali* and a recent international review of the genus *Salsola* section *Salsola sensu lato* conducted by Rilke reclassified all Australian populations as *S. tragus* (Rilke 1999a; Wilson 1984). However, genetic work (Ryan and Ayres 2000) has indicated that the revised classification by Rilke (1999a) was not complete, which is understandable given the difficulties inherent in classification of *Salsola* species discussed above. For the purposes of this review, the classification of Australian species

of the *Salsola* genus developed by Rilke (1999a) is utilised, although this classification system is not accepted in all States of Australia (Australian Plant Name Index 2005; Western Australian Herbarium 2007). However, this review incorporates literature on those species with which *S. tragus* is commonly confused, as well as other common weed species of the *Salsola* genus. Further, when discussing previous research on a given species of the *Salsola* genus, the species name used by the author in question is not changed, even where later research has indicated that the species has been reclassified.

### ***Salsola tragus*** **in Australia**

*Description*

*Salsola tragus* is the only species of the *Salsola* genus present in Australia. However, it has been noted that within Australia *S. tragus* has developed a broad diversity of polymorphic forms; more so than in Africa or America where it is also an invasive weed (Rilke 1999a). The following key devised by Rilke (1999a) differentiates between the subspecies of *S. tragus* found in Australia.

Flowers small, 2-3.4 mm long, anthers (0.6) 0.8-1.4 (1.6; 1.9) mm long, fruit without a column or column <1.5 mm, column clearly shorter than the wing.

> Plant ± densely branched, leaves ± succulent, glabrous or hairy, fruit at least in major part of the fructification with membranous wing, plant of the interior or the seacoast.
>
> A: subsp. ***tragus*** L.
>
> Plant slightly branched; leaves succulent, often glabrous; all or at least the major part of the fruits enclosed in the bract and bracteoles, fruits predominantly without a wing; plant of the southern Mediterranean seacoast.
>
> B: subsp. ***pontica*** (Pall.) Rilke.

Flowers large, 7-10 mm diameter, anthers 1.2-1.9 mm long, fruits with 2-3 mm high column, column nearly as long as the wing; restricted to the northern regions of Australia.

C: subsp. ***grandiflora*** Rilke.

*Salsola tragus* subsp. *tragus*, the most common weedy form in Australia, is usually an erect, rounded herb with a diameter of up to 0.6 m, although it can have a prostrate

growth form and plants with a diameter up to 2 m have been observed (Mitchell and Wilcox 1988; Wilson 1984) (Figure 2.1, 2.2).

Figure 2.1: *Salsola tragus* subsp. *tragus* at the flowering stage (left) and senesced, mobile plants in a field containing barley stubble (right), in the district of Morawa, Western Australia.

There are many variants of *S. tragus* in Australia that are morphologically distinct but are not classified as separate subspecies. Forms with fruits congested in globular or ovoid spikes have been noted and previously classified as *S. kali* var. *strobilifera* Benth. (Wilson 1984). However, it has since been suggested that this variation is the result of a simple mutation that arises in several species of the *Salsola* genus and should not be recorded as a distinct subspecies (Rilke 1999a). A significant polymorphism observed in populations of *S. tragus* in Australia includes differences in the growth form, from annual to perennial. Most populations are annual, but Rilke (1999a) identified a population on the York Peninsula where single plants appeared to survive for 2 to 3 years. Annual *S. tragus* populations complete the juvenile vegetative growth stage before entering the reproductive stage and reaching senescence, but within the perennial population, vegetative growth continued during the reproductive phase. Other polymorphisms included the amount of stem hair on mature plants, succulence of leaves, bract size, exposure of inflorescences, branch length, anther size, fruit size, general fruit structure and others, which are described in detail by Rilke (1999a).

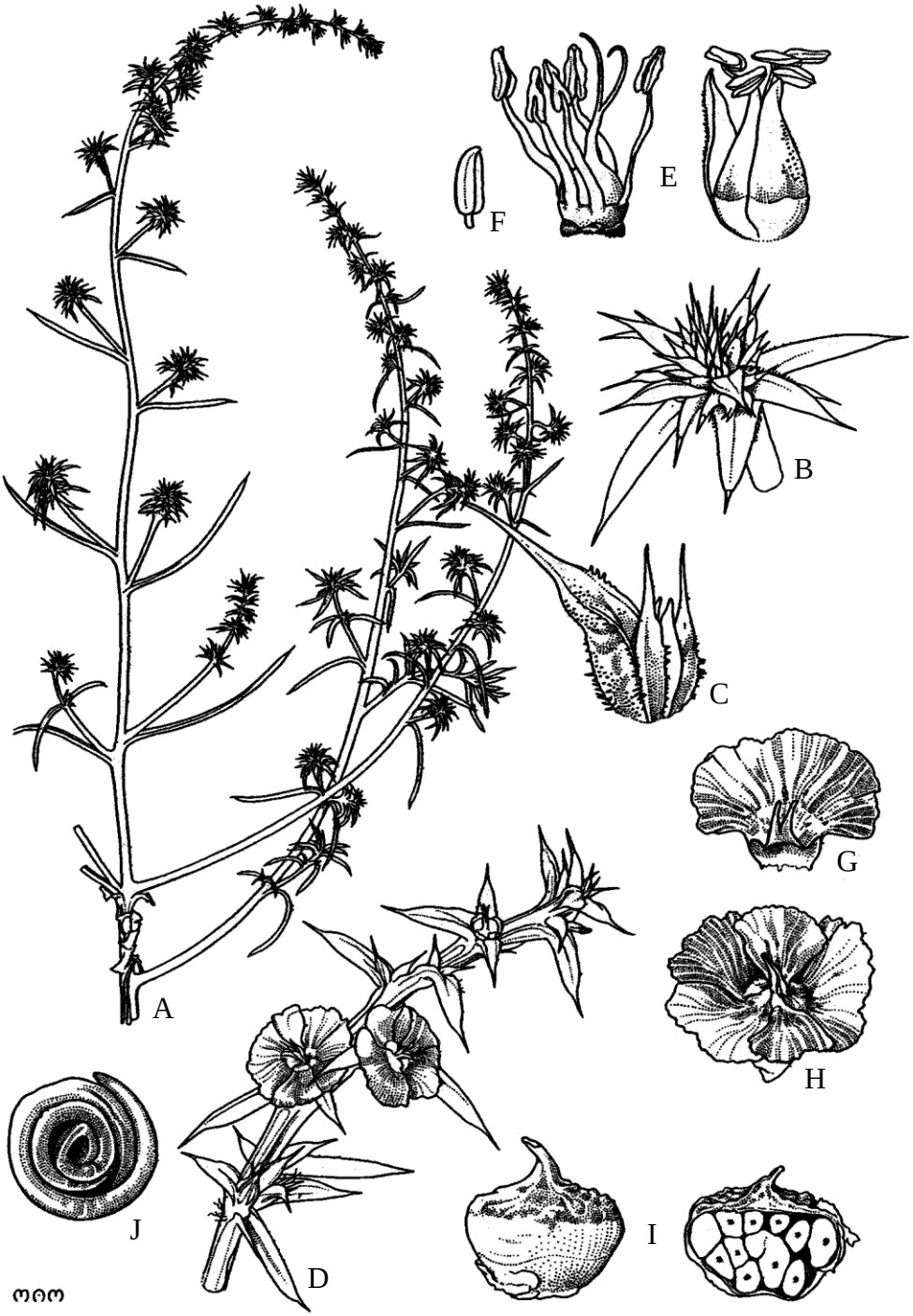


Figure 2.2: *Salsola tragus* subsp. *tragus*. The number next to each object indicates the magnification. A, branch x 1; B, terminal glomerule x 5; C, bud in bract and bracteoles x 15; (A-C, Meekatharra, W.A., A. Mitchell, Perth) D, branch x 2.5; E, flower, with and without tepals x 15; F, anther x 20; G, fruiting tepal x 7; H, fruiting perianth x 5; I, fruit, entire and L.S. x 15; J, seed x 15 (D-J, Meckering, W.A., P. Wilson, Perth) (Wilson 1984). Artist: Margaret Menadue. Reproduced with permission from Flora of Australia, Volume 4.

*Australian distribution*

*Salsola tragus* is widely distributed throughout Australia, with the exception of Tasmania (Mitchell and Wilcox 1988) (Figure 2.3). *Salsola tragus* subsp. *tragus* is by far the more common variant and is found in a very broad range of habitats throughout mainland Australia. *Salsola tragus* subsp. *grandiflora* is found in isolated populations in the northern regions (Kimberly) of Western Australia, as well as the Northern Territory and Queensland (Rilke 1999a). *Salsola tragus* subsp. *pontica* exists in littoral zones on the north-east and south-west coast of Australia (Rilke 1999a). The division of subspecies is not indicated on the herbarium map in Figure 2.3 as the classification of subspecies is not recognised in all Australian States.

*Salsola tragus* is most abundant in disturbed habitats (preferably with alkaline, saline soils or limestone banks), including waste areas, roadsides, rain-fed cropping or pastoral agricultural environments and mine sites (Mitchell and Wilcox 1988; Mussell and Stewart 2004; Naidu and Harwood 1997; Smith 1960). It is often one of the first species to volunteer on disturbed sites. Naidu and Harwood (1997) found it invading the spoils at Goonyella mine in the Bowen Basin (Queensland) in spite of very low rainfall and extreme salinity. In disturbed areas it may play a valuable role in the revegetation process by volunteering on highly degraded soils and preventing erosion, adding humus, feeding soil microbes and improving soil aggregate formation and water infiltration (Naidu and Harwood 1997). *Salsola tragus* is also found in natural environments such as high and low rainfall woodlands, shrub lands, grasslands, sandy sites, semi-deserts and coastal habitats (Beadle 1981; Mitchell and Wilcox 1988; Rilke 1999a). However, usually some disturbance of native vegetation is required before *S. tragus* can invade natural environments (i.e. rabbits burrowing or intermittent floods) (Eldridge et al. 2006; Westbrooke and Florentine 2005).

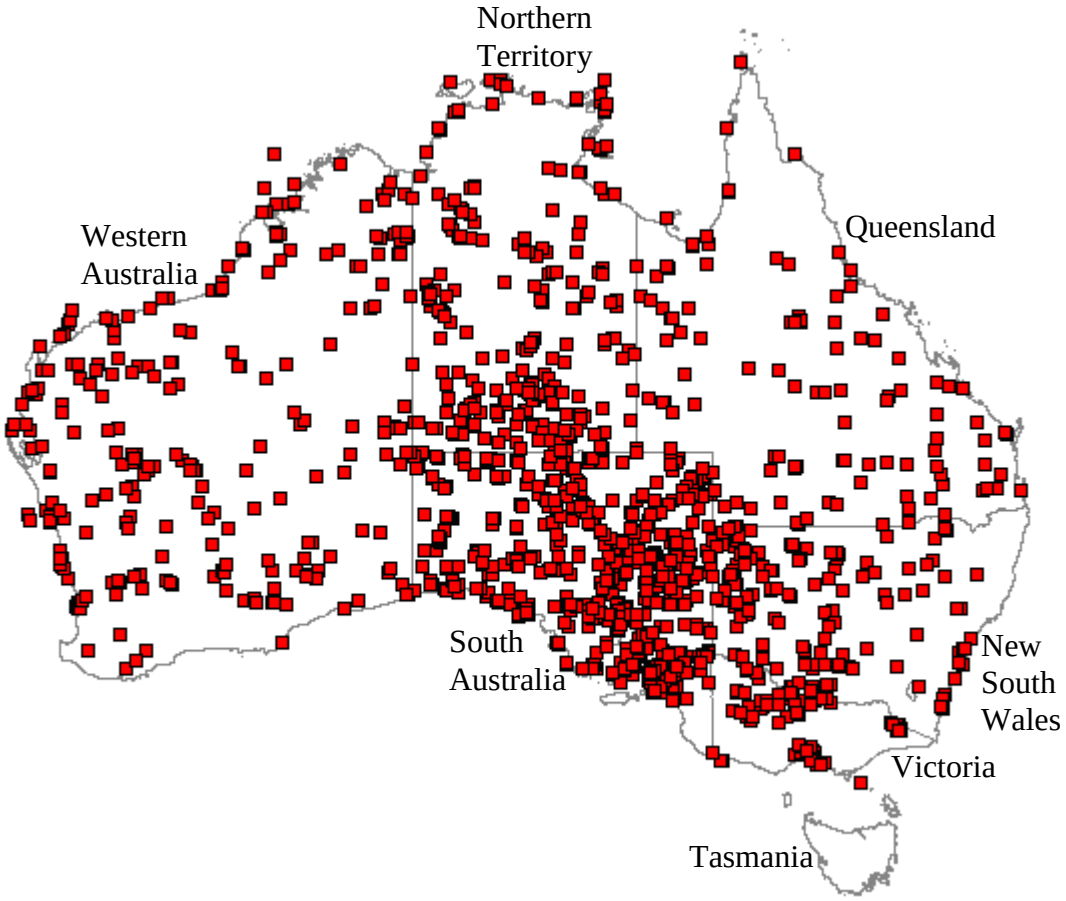


Figure 2.3: Herbarium records of *S. tragus* in Australia. Image used with the permission of Australia's Virtual Herbarium, via Centre for Plant Biodiversity Research, Council of Heads of Australian Herbaria (Australia's Virtual Herbarium 2004). Accessed on Friday, 13 August 2004.

*Climatic requirements*

*Salsola tragus* is found within all the major climatic regions of Australia. It is found throughout southern Australia, from the mediterranean climate region in southern Western Australia to temperate New South Wales and Victoria (Bureau of Meteorology 2007a; Rilke 1999a; Wilson 1984). The arid, desert interior region of Australia, which receives 100-400 mm of rainfall per year and has very high evaporation rates, likewise supports *S. tragus*. This weed is also found in the northern regions of Australia, although it is less common in the monsoonal, tropical regions of Queensland, the Northern Territory and Western Australia. This weed is not found in Tasmania, which experiences a temperate climate (Figure 2.4).

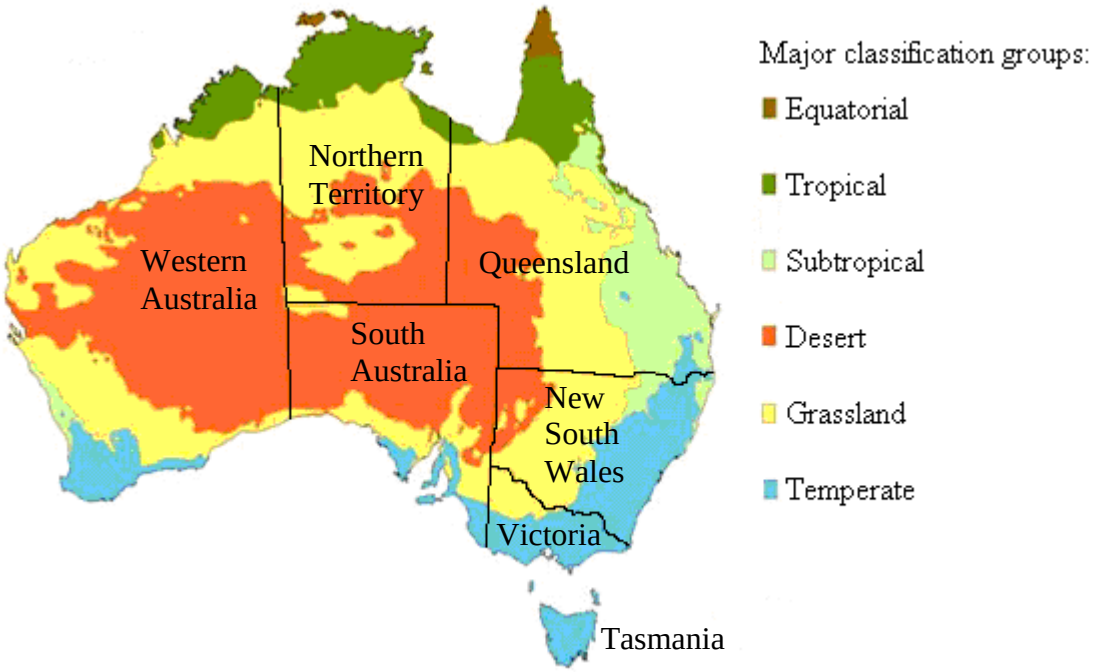


Figure 2.4: Major climate classification groups in Australia. From the Bureau of Meteorology (Bureau of Meteorology 2007a). Accessed on Monday, 21 May 2007.

*History*

The *Salsola* genus probably evolved in the arid regions of Europe and Asia (Rilke 1999b; Young 1991). *Salsola tragus* subsp. *tragus* is still distributed over this area (Rilke 1999a) and has since migrated to Australia (possibly with human assistance), where it was established before 1770 (Smith et al. 1980). *Salsola tragus* migrated to America in the late 1870s and was found in Africa in 1885 (Rilke 1999a; Young 1991).

It is probable that this species arrived in Australia shortly before European invasion; introduced by earlier visits of other cultures to Australia or through long distance dispersal (Kadereit et al. 2005). The source of *S. tragus* in Australia has not been investigated. Most authorities agree that it is an introduced species, although there is argument that the genus may be native or naturalised in Australia (Australian Plant Name Index 2005; Bean 2007; Hussey et al. 1997). *Salsola tragus* (labelled as *S. kali* L.) was first collected in Australia in 1770 by Banks on the east coast (Bay of Inlets and Endeavour River Valley, Queensland) (Smith et al. 1980). Further collections were made by Robert Brown (1810) in 1802-1805, who identified two species in the *Salsola* genus, *S. australis* and *S. macrophylla*. *Salsola australis* is described as a fleshy, branched herb and *S. macrophylla* as an erect, woody plant (Brown 1810). *Salsola*

*australis* was classified as *S. tragus* by Rilke (1999a). *Salsola macrophylla* is not a separate species, but rather any plant with succulent leaves and bracts (diameter >2 mm), suggested to be an environmentally induced variation possibly caused by high salinity levels (Rilke 1999a). *Salsola tragus* subsp. *grandiflora* is endemic to Australia (Rilke 1999a). The plant was described in 1870 in New South Wales and Victoria, and was recorded in all States on the mainland by 1921 (Bentham 1870; Domin 1921; Rilke 1999a).

*Species variability*

There is likely a high degree of genetic variability between *S. tragus* populations in Australia. Observed morphological diversity as well as environmentally induced variation and adaptation to varying habitats across Australia all indicate the presence of genetic diversity in *S. tragus*. Rilke and Reimann (1996) found a high level of genetic variation within populations of *S. tragus* from Israel and Tunisia, which accounted for variation in fruit morphology, stem colour and stem or leaf hairs. Variation in salt tolerance, ion relations, succulence, growth forms, colour, leaf size and other morphological traits appeared to be environmental (Rilke and Reimann 1996). Ryan and Ayres (2000) found that Californian *S. tragus* actually consisted of *S. tragus* and a morphologically similar, but genetically distinct variety that was not positively identified. However, in spite of the morphological variation observed in Australian populations, no attempt has been made to further subdivide the species or differentiate between genetic and environmentally induced variation (Rilke 1999a; Wilson 1984). It is likely that more subspecies of *S. tragus* are in Australia than the three currently recognised. Alternatively, given the confusion in the classification of species within the *Salsola* genus, it is possible that the morphological variation indicates the presence of other *Salsola* species in Australia. Investigation of the genetic variation within Australian populations of *S. tragus* may result in improved classification of the *Salsola* genus in Australia, improved quarantine restrictions on the importation of *Salsola* species, determination of the origin of Australian *S. tragus* and improved weed management.

## Life cycle

### *Germination*

Seeds of the *Salsola* species growing as weeds in agricultural areas generally have very broad germination requirements. *Salsola kali* plants produce seeds that are simple, with no stored energy reserves or complex coverings to offer protection (Dwyer and Wolde-Yohannis 1972; Evans and Young 1972; Young 1991). A seed contains a coiled, embryonic but fully differentiated plant (Wallace et al. 1968; Young 1991). This plant is thought to be slightly immature, which results in a period of after-ripening before germination occurs (Young 1991). Germination occurs when the seed imbibes water, and the cells of the embryonic plant elongate. No cell division occurs during this process. The elongated cells cause the seedling to uncoil, breaking the seed coat and forcing the radicle into the soil. If the radicle encounters and absorbs sufficient water, the cotyledons expand and cell division commences (Wallace et al. 1968; Young 1991). Active chloroplasts in the cotyledons allow photosynthesis to start immediately (Crompton and Bassett 1984). As a result, the seedling can begin to emerge 30-45 minutes after the seed is exposed to moisture and the entire process of germination and establishment can occur in less than two hours in ideal conditions (Dwyer and Wolde-Yohannis 1972; Wallace et al. 1968). Similar germination characteristics and aspects of seed biology were found in *S. vermiculata* and *S. pestifer* (Creager 1988; Crompton and Bassett 1985).

### *Seed burial*

Seed burial is not necessary for germination, but increases the likelihood that a seedling will successfully establish. Germination of *S. vermiculata, S. kali, S. paulsenii, S. iberica* and *S. rigida* Pall. occurred in the constant presence or absence of light (Al-Charchafchi et al. 1987; Creager 1988; Sankary and Barbour 1972; Wallace et al. 1968). Likewise, the presence or absence of litter (on disturbed soil) made little difference to *S. kali* germination (Evans and Young 1970). Establishment was reduced by removal of the fruiting perianth, probably because this structure gives the uncoiling seedling something to push against to allow the radicle to enter the soil (Sankary and Barbour 1972). However, removal of the fruiting perianth makes a seed significantly smaller, which would facilitate burial (Evans and Young 1970). Overall germination percent (measured as successful establishment) was generally slightly lower on the soil surface

and increased with burial to a depth of 3-7.5 cm for *S. paulsenii*, *S. kali* and *S. iberica* (Wallace et al. 1968; Young and Evans 1979).

Burial or the presence of litter normally benefits germination because it reduces temperature extremes and increases moisture retention compared with bare soil. However, germination for weedy species of the *Salsola* genus is rapid, temperature requirements for germination are broad and the moisture requirements are low. As a result, the seedlings can become rooted during the shorter periods of optimum temperature and moisture that occur on bare soil compared to litter (Dwyer and Wolde-Yohannis 1972; Evans and Young 1970; Young 1991). Seeds germinating on the surface of compacted soil often desiccated because the radicle could not penetrate the soil surface. However, where the soil had been recently disturbed or the seed was firmly pushed against the soil surface, the spiralling action of the uncoiling embryo was sufficient to push the radicle into the soil and allow successful establishment (Wallace et al. 1968; Young and Evans 1979). The ability to germinate and establish on bare soil is a necessary adaptation in an early successional species such as those found in the *Salsola* genus (Evans and Young 1970).

*Plant growth*

Following seedling establishment, *S. kali* in Australia enters an initial growth phase with soft, bright green leaves that are round in cross-section. The leaves change to a lighter green and become stiff, flatter and taper to a spine 1-4 mm long. Flowers develop between the leaves and stem, towards the end of the branches. Anthers vary in length from 0.4 to 1.4 mm. A seed case develops after seed set, which consists of a single seed surrounded by 5 horizontal, papery wings 5 to 11 mm wide (Mitchell and Wilcox 1988; Wilson 1984). The wings are of equal size, although seeds with no wings are also produced (Mitchell and Wilcox 1988). At senescence the above ground proportion of the plant can break free of the root system and disperse by wind (Mitchell and Wilcox 1988). Plants reach a maximum size of approximately 2 m in diameter (Mitchell and Wilcox 1988) (Figure 2.5). *Salsola kali* in the USA has a similar growth form, although plants can grow to the size of a small car, and produce flowers with 3 wings and 2 reduced wings (Rilke 1999a; Young 1991).

Figure 2.5: The author, next to senesced *S. tragus* found in Fresno, California (left) and in Yalgoo, Western Australia (right). Each plant was the largest identified after several days of touring each State.

*Pollination*

Species of the *Salsola* genus appear to be wind pollinated, and can produce a very large number of seeds with high viability. *Salsola pestifer* and *S. kali* are wind pollinated and viable seed is produced autogamously or allogamously in late summer (Crompton and Bassett 1985; Young 1991).

*Reproduction*

Seed production is high, with large *S. kali* plants in North America producing an average of 250 000 seeds with 95-100% viability (Dwyer and Wolde-Yohannis 1972; Evans and Young 1972; Young 1991). *Salsola iberica* in the USA produced an estimated 17 671 to 151 552 seeds per plant, and seed viability was greater than 90% (Fowler et al. 1988; Stallings et al. 1995). *Salsola paulsenii* plants in the USA could produce over 200 000 seeds per plant with greater than 90% viability (Evans and Young 1980; Young and Evans 1979). *Salsola pestifer* plants in Canada only produced 2000 seeds per plant with 44% viability (Crompton and Bassett 1985). Seed production is closely correlated to plant size in several species, and plant height can vary from a few centimetres to approximately 2 m tall (Crompton and Bassett 1985; Stallings et al. 1995; Young 1991) (Figure 2.4).

*Short term (<1 yr) dormancy*

According to the classification system developed by Baskin and Baskin (2004), seeds of several *Salsola* species experience non-deep physiological dormancy, where a strict temperature requirement for germination is gradually removed as the seeds progress

from a dormant to non-dormant state over several months. This germination inhibition is likely due to temperature-dependent after-ripening requirements. Young (1991) stated that in the northern regions of the USA, germination of *S. kali* seed predominately occurred at a constant temperature of 24 to 27$^{o}$C during the winter following seed production. This temperature range did not commonly occur in winter and so the plant was protected from germinating in an unsuitable season. Once after-ripening was complete and the seeds were fully matured 6 months later (during spring), germination occurred at a temperature range of -2 to 43$^{o}$C. Further, the temperature did not need to remain constant for germination to occur, although germination rates were generally higher and more rapid in moderate to warm temperatures (Young 1991). Narrow initial temperature requirements, followed by broad temperature requirements after a period of after-ripening was noted in *S. kali*, *S. paulsenii* and *S. iberica* seeds (Allen 1982a; Dwyer and Wolde-Yohannis 1972; Evans et al. 1982; Young and Evans 1972; 1979).

Alternatively, short term dormancy in some species of the *Salsola* genus may be chemically induced, which was investigated for populations of *S. kali* and *S. vermiculata* found growing in coastal habitats, in the line of decaying seaweed and debris at high tide mark (strandline populations). Ignaciuk and Lee (1980) noted that the germination of *S. kali* seeds was limited by the fruiting perianth. They concluded that the gradual degradation of the fruiting perianth or leaching of the chemical inhibitors it may contain (over a period of several months) may result in an increase in germination. Likewise, *S. vermiculata* seeds had a higher percentage germination when the fruiting perianth was removed (Osman and Ghassali 1997; Sankary and Barbour 1972). However, the effect of removing the fruiting perianth was only examined in laboratory conditions. Germination of green *S. vermiculata* seed, collected weeks before dispersal, was inhibited by the perianth, but was high when the perianth was removed (Creager 1988). The predominant role of the bracts may be to inhibit germination of immature seeds attached to the plant, especially if these seeds have not yet developed physiological dormancy. Whether dormancy results from temperature dependent after-ripening requirements or chemical inhibition from the fruiting perianth, dormancy is short-term, enabling the bulk of the seed to germinate during favourable conditions for plant growth in the year following seed production.

*Long term (>1 yr) dormancy*

Species of the *Salsola* genus that grow as agricultural weeds produce seeds with very short lived seed banks. *Salsola iberica* in the Pacific Northwest region of the USA produced seeds that predominately germinated in the year following seed production, and very little viable seed remained after the second year in field conditions (Young et al. 1995). Likewise, the majority of viable *S. kali* seeds germinated in the year following seed production and residual seeds from previous years had very low germination rates (Evans and Young 1972). *Salsola paulsenii* seeds in field conditions in Nevada, USA, experienced 65% germination in the first year and very few seeds remained by the third year (Chepil 1946; Evans and Young 1980). Sankary and Barbour (1972) found that *S. vermiculata* seeds in laboratory conditions remained viable for less than one year, although Creager (1988) found that *S. vermiculata* seeds could remain viable for over two years. *Salsola iberica* seed stored for over a year in laboratory conditions rapidly lost viability (Fowler et al. 1988).

Dormancy of seeds that remain attached to the mature plants has not been investigated. Young (1991) noted that if *S. kali* plants do not move vigorously, most of the seeds remain attached and lose viability within months. However, there are no data for loss of viability of attached seeds over time, or loss of viability of attached seeds compared to seeds that are shed from mature plants.

*Seed dispersal*

Species of the *Salsola* genus commonly engage in broad scale seed dispersal (Mallory-Smith et al. 1993; Schmidt and Reeves 1989; Stallings et al. 1995). Senesced plants, retaining viable seed, break free of the root system through the degradation of a specialised layer of cells at the base of the stem (Evans and Young 1972; Mallory-Smith et al. 1993; Mitchell and Wilcox 1988; Stallings et al. 1995; Warren 2001; Young 1991; Young and Evans 1979). The senesced plants are spherical or hemispherical, with springy branches. This form allows the wind propelled plants to tumble considerable distances, bouncing over physical obstacles (Crompton and Bassett 1985; Young 1991). Seed dispersal is affected by the distance and direction of plant movement and the time and rate of seed shedding.

The long distance seed dispersal events are likely to have a profound effect on the population dynamics of *Salsola* species, and will influence invasion rate, competition and the genetic structure of populations (Nathan and Muller-Landau 2000). Establishment of distant populations, or even maintenance of genetic heterogeneity of spatially separated populations would enhance invasion speed (Cousens and Mortimer 1995). Seeds that can escape the density dependent mortality of the parent population (predation, intra/interspecies competition) are likely to be more successful (Fuentes 2002; Nathan and Muller-Landau 2000; Vander Wall and Longland 2004). The degree of seed dispersal varies widely between species of the *Salsola* genus and so the impact on population dynamics will be just as variable.

**Environmental adaptations**

*Salsola tragus* in Australia is most common in harsh environments (Mitchell and Wilcox 1988; Mussell and Stewart 2004; Naidu and Harwood 1997). In the USA, *S. kali* plants even established at ground zero in areas used for atomic weapons testing, and other areas with solid or liquid toxic waste (Johnson et al. 1994; Warren 2001; Young 1991). Moreover, *S. tragus* and related species of the *Salsola* genus are early successional species. They establish dense populations in disturbed habitats, but decline if the area does not experience frequent disturbance events (Allen 1982b; Hironaka and Tisdale 1963; Lee and Ignaciuk 1985; McLendon and Redente 1992; Wali 1999). Schmidt and Reeves (1989) found that while *S. kali* was initially the dominant pioneer species following disturbance in western Colorado, the population declined and became stunted two to three years after colonising the site, even while still growing as a monoculture and producing seed on an annual basis.

*Salinity tolerance*

Chenopodiaceae is the family in which salt tolerance is most widespread (Flowers et al. 1986; Ranwell 1972). Forty four of the 100 genera found in this family (312 species), including the genus *Salsola*, consist of species that either tolerate or require saline environments (Flowers et al. 1986). The growth of several species of *Salsola* is stimulated by low levels of salinity (Flowers et al. 1986; Rilke and Reimann 1996). Rilke and Reimann (1996) found that at a salinity concentration of 100 mol $m^{-3}$ NaCl, the biomass and fruit production of *S. kali* subsp. *kali* L. and subsp. *tragus* plants was greater than that of plants in non-saline conditions. At salinity concentrations above 200

mol $m^{-3}$ NaCl, growth was reduced, but the plants survived to produce seed at salinity concentrations up to 800 mol $m^{-3}$ NaCl. In comparison, wheat varieties bred for salt tolerance suffer yield reductions at 125 mol $m^{-3}$ NaCl (Ashraf and Khanum 1997). Several other authors have noted an increase in germination, growth, and reproduction in *S. kali*, *S. kali* subsp. *tragus* and *S. iberica* plants in moderately saline conditions (Fowler et al. 1988; Ignaciuk and Lee 1980; Lee and Ignaciuk 1985; Reimann and Breckle 1995; Rozema et al. 1982).

It is difficult to determine if these species of the *Salsola* genus are true halophytes that thrive on saline soils, or merely species that can tolerate saline conditions. For example, *S. kali* can tolerate high salinity levels, but can grow just as well under non-saline conditions, and may not necessarily benefit from the presence of salt (Eshel 1985; Eshel and Waisel 1984). While many studies have found that biomass of several species of the *Salsola* genus increased at moderate salinity levels, it is difficult to determine if this increased biomass resulted from a true growth stimulation (increased organic matter) rather than increased ion and water content. Reimann and Breckle (1995) and Rilke and Reimann (1996) did not address this issue. Flower et al. (1988) noted an increase in dry biomass for *S. iberica* plants growing at salinity concentrations of 10.5-18.2 dS $m^{-1}$. They concluded that approximately 15-23% of the increase in biomass resulted from the increase in ion content (sodium and chloride), and the remainder was presumably due to an increase in organic matter production. However, Eshel (1985) noted growth stimulation of *S. kali* plants at moderate salinity levels and concluded that the added biomass was mainly due to the increase of ions and turgor enhanced growth. However, *Salsola* species are $C_4$ plants and unlike $C_3$ plants, they require sodium as a micronutrient to support normal growth (Flowers et al. 1986). The increased availability of sodium at moderate salinity levels may be the cause of the observed growth stimulation (Flowers et al. 1986).

Salt induced growth stimulation or salt tolerance has not been investigated in Australian populations of *S. tragus*, but has implications for the management of this species. Salt tolerant species in Australia can be used in the initial stages of mine site rehabilitation while salt is leaching from the mine site tailings, and as forage crops in saline agricultural regions (Naidu and Harwood 1997; Rogers et al. 2005; Semple et al. 2006). Internationally, species of the *Salsola* genus are used for both purposes (Toderich et al.

2002; Wali 1999). However, saline regions in Australian agriculture are expanding (Kingwell et al. 2003), which may result in the spread of salt tolerant weeds. Growth and productivity of most crop and pasture species is reduced in saline soils (O'Connell et al. 2006; Turner and Asseng 2005). Reduced crop competitiveness combined with growth stimulation of *S. tragus* in saline conditions may allow this weed to become increasingly competitive and prevalent in the broad scale cropping regions. The salinity tolerance of *S. tragus* in Australia will determine the extent to which this plant is useful in rehabilitation projects and detrimental as an agricultural weed.

*Water use*

*Salsola* species often grow in arid environments, and survive by requiring very little water for germination and growth. Germination of several species (*S. rigida*, *S. iberica* and *S. kali*) occurred in the lab after seeds received the equivalent of 0.3 cm of rainfall (Al-Charchafchi et al. 1987; Dwyer and Wolde-Yohannis 1972; Young 1991). Seedlings of *S. rigida* and *S. kali* have the ability to pause growth if they desiccate, and then resume normal growth when moisture is again available (Wallace et al. 1968). *Salsola rigida* seedlings less than 6 hours old were dried and then hydrated twice, and still continued to grow after being hydrated for the third time (Al-Charchafchi et al. 1987).

While species of the *Salsola* genus can germinate and grow in arid conditions with low moisture availability, they are also highly effective at removing soil moisture. *Salsola iberica* plants in Lind, Washington State, had a root system that covered a lateral distance of 1.5 m and a vertical distance of more than 1.8 m (Pan et al. 2001). Single *S. iberica* plants were capable of removing 70 L of soil moisture while growing in a spring wheat crop and 100 L in the fallow period following crop harvest (Schillinger and Young 2000). Rooting systems extending over a metre are a common trait in species of the *Salsola* genus and have also been found in *S. vermiculata* and *S. pestifer* (Creager 1988; Crompton and Bassett 1985). However, while species of the *Salsola* genus can utilise higher levels of water when it is available, water use efficiency is adaptable and increases to allow survival in arid conditions. Dwyer and Wolde-Yohannis (1972) found that in soils at field capacity, *S. kali* required 181 mL of water to produce 1 g dry biomass. In dry soils experiencing a single simulated rainfall event of 3 mm, water use efficiency increased and plants could produce 1 g of dry biomass with 76 mL of water.

Fowler and Hageman (1978) also noted that the water use efficiency of *S. iberica* and *S. paulsenii* was very high and improved with reduced water availability. As expected for a $C_4$ plant, *S. iberica* (or *S. paulsenii*) was found to require half to one third as much water per unit of dry matter produced than $C_3$ pasture species such as alfalfa (*Medicago sativa* L.), flax (*Linum usitatissinum* L.) or bromegrass (*Bromus* sp.) (Briggs and Shantz 1914).

The high water use efficiency found among these $C_4$ *Salsola* species gives them a competitive advantage over less efficient species in dry conditions. *Salsola kali* could successfully colonise arid regions because it could out compete other species for water (Allen 1982b). This high water use efficiency and increased competitive ability indicate why *S. iberica* is such a successful in-crop weed in the dry-land cropping system in Washington State (Young 1986; 1988). Likewise, high but adaptable water use efficiency in *S. tragus* is likely to be the reason why this $C_4$ plant is such a widespread summer weed in the arid to semi-arid Australian cropping system.

*Stress metabolites*

The production of stress metabolites, which is a feature of Australian native species (as well as *S. kali* in Queensland), is an adaptation that allows plants to endure harsh environmental conditions like salinity, drought and temperature extremes (Naidu 2003; Naidu et al. 1987; Poljakoff-Mayber et al. 1987; Storey et al. 1977). Glycinebetaine is the stress metabolite commonly found in species of the *Salsola* genus (Naidu et al. 1987; Wyn Jones and Storey 1981). In conditions of water stress and high temperatures, *S. kali* had 51-70 µmol of glycinebetaine $g^{-1}$ of fresh plant tissue (Poljakoff-Mayber et al. 1987; Smirnoff and Stewart 1985). In saline conditions, *S. kali* plants have up to 25% of their nitrogen invested in stress metabolites (Pakeman and Lee 1991a). These compounds may confer salinity tolerance by acting as cytoplasmic osmotica, i.e. balancing the low osmotic potential of vacuoles where sodium chloride is stored when plants are growing in saline conditions (Smirnoff and Stewart 1985; Stewart and Lee 1974; Storey et al. 1977). They appear to stabilise enzymes and proteins to allow them to maintain normal activity in stressful conditions (Paleg and Aspinall 1981; Smirnoff and Stewart 1985). This allows plants to more quickly resume growth in favourable conditions, enhancing competitive ability (Smirnoff and Stewart 1985).

The stress metabolites produced by species of the *Salsola* genus are nitrogen based compounds (Wyn Jones and Storey 1981). As such, the degree of stress tolerance is related to nitrogen availability. *Salsola kali* and *S. iberica* plants were the dominant species in disturbed habitats when nitrogen was freely available and they could produce ample levels of stress metabolites, but stress metabolites and the related competitive ability declined with declining nitrogen (Allen 1982b; Lee and Ignaciuk 1985; McLendon and Redente 1992; Pakeman and Lee 1991a; 1991b; Ranwell 1972; Storey et al. 1977). The potential for high water use efficiency in *S. iberica* and *S. paulsenii* is positively correlated to nitrogen availability (Fowler and Hageman 1978). Pakeman and Lee (1991b) found that *S. kali* plants in a strandline environment produced the same amount of glycinebetaine whether nitrogen was abundant or deficient. As a result, growth and reproduction were reduced in nitrogen deficient conditions as nitrogen was diverted from reproduction, to the production of stress metabolites (Pakeman and Lee 1991b; Storey et al. 1977).

The relationship between stress tolerance (leading to competitive ability) and nitrogen availability explains why several species of the *Salsola* genus, like *S. iberica*, are successful agricultural weeds (Young 1988). The application of nitrogen fertilisers to the crop would increase the competitive ability of *Salsola* weeds in dry-land farming systems that experience moisture stress. Stress metabolites have not been investigated in *S. tragus* populations. However, in Australia *S. tragus* is a successful summer weed, especially in stressful environments. While it does not successfully compete within crops, as a summer weed it removes soil nitrogen during the summer fallow that could have been utilised by the following winter crop (Osten et al. 2006). Nitrogen removed by *S. tragus* (as opposed to other summer weeds) may have a more significant impact on crop yield because the plants become mobile at senescence and often move to the edge of the field or escape entirely, effectively removing nitrogen from the system.

*Autotoxic effects*

One possible reason why some populations of *Salsola* species decline in the absence of disturbance is that they are auto-toxic, i.e. produce allelopathic compounds that adversely affect their own growth more than the growth of other species. Lodhi (1979) observed a reduction in the growth of *S. kali* plants when powdered *S. kali* leaf litter was added to the soil, or when plants were irrigated with water that had previously been

passed over freshly harvested, leafy *S. kali* branches. Schmidt and Reeves (1989) found that leachates from *S. kali* roots depressed the growth of *S. kali* plants but did not affect other species, and concluded that autotoxic chemicals may have resulted in stunted *S. kali* populations. Osman and Ghassali (1997) found that the leachate from the fruiting bracts of *S. vermiculata* inhibited the germination of *S. vermiculata* by 20% and the germination of *Atriplex halimus* L. by 8%. However, none of the above mentioned experiments examined autotoxicity in normal field conditions. Research with allelochemicals in natural environments indicates that the chemicals are at varied concentrations, and are influenced by edaphic factors (e.g. temperature and moisture) and the soil microbial population (Bruce 2003; Fisher 1993; Harper and Lynch 1982). Further work with experiments designed to reflect field conditions are required to determine if the toxic compounds produced by species of the *Salsola* genus inhibit their own growth.

Autotoxicity is apparent in several crop and weed species, and contributes to population regulation (Alias et al. 2006; Kraus et al. 2002; Oueslati et al. 2005). However, the evolutionary advantage gained by *Salsola* species from restricting their own growth is not readily apparent. It is conceivable that toxic compounds are produced in an attempt to reduce soil pathogens, but there is evidence that mycorrhizal fungi are pathogenic on *S. kali* and these toxins had no effect on the mycorrhizal fungi found in the soil with *S. kali* (Schmidt and Reeves 1989).

*Pathogenic mycorrhizal fungi*

Mycorrhizal fungi colonise plants in a symbiotic relationship where they provide the plants with nutrients in exchange for carbohydrates, and possibly influence water uptake, suppress root pathogens and improve soil structure (Smith and Read 1997; Wright and Upadhyaya 1998). While mycorrhizal fungi colonise *S. kali* plants, there is some indication that they act as a pathogen. Allen et al. (1989) found that following invasion by mycorrhizal fungi, the roots of *S. kali* seedlings subsequently turned brown and died, leading to the eventual death of approximately 25% of the seedlings. This incompatible response on the part of *S. kali* clearly inhibited its own growth. However, the fungus could extract carbon from the roots and complete its life cycle before the root died, allowing it to multiply and move on to invade other plants (Allen et al. 1989). The density and percent cover of *S. kali* plants was reduced by approximately 30% following

inoculation of the soil with mycorrhizal fungi (Allen and Allen 1988). Likewise, O'Connor et al. (2001) noted that mycorrhizal fungi invaded *S. kali*, but probably did not contribute to the uptake of phosphorus by the plant. Mycorrhizal fungi are at relatively low levels in soil following a single disturbance event, as the removal of vegetation retards their growth (Smith and Read 1997). As mycorrhizal fungi can survive and expand in the presence of *S. kali*, the gradual increase in fungal populations may result in the concomitant decline of *S. kali* populations. In areas of constant disturbance like agricultural fields, the mycorrhizal population may be prevented from increasing to the point where they affect colonising species of the *Salsola* genus.

Whether or not mycorrhizal fungi act as a pathogen against *Salsola* species, the gradual increase of mycorrhizal fungi levels would still reduce the fitness of *Salsola* species against species that can utilise a mycorrhizal symbiosis to obtain nutrients (Allen and Allen 1988; Allen et al. 1989; Wali 1999). Given that *Salsola* species require high levels of nitrogen to create stress metabolites and tolerate stressful environments, reduction in nitrogen availability through competition with mycotrophic species would reduce competitive ability and environmental tolerance of *Salsola* species (Pakeman and Lee 1991b). However, Allen (1989) found that inoculating soil with mycorrhizal fungi or removing the *S. kali* population from a disturbed area did not improve the growth of later successional species that eventually replace *S. kali*. This indicates that species of the *Salsola* genus that colonise disturbed areas play an important role in modifying the environment to allow other species to establish (Allen 1989).

### *Salsola tragus* as an agricultural weed

Carbonised seeds of an unidentified species of the *Salsola* genus were found in the oldest agricultural sites in southern Eurasia (Young 1991), indicating that species of this genus have existed in conjunction with agricultural systems for thousands of years. For a large proportion of the year, agricultural land is covered by a layer of very short vegetation, either stubble from a harvested crop or grazed pasture plants. Once they reach senescence, several agricultural weed species of the *Salsola* genus have the ability to travel vast distances over areas with little vegetation, to rapidly exploit disturbed areas (Mallory-Smith et al. 1993; Young 1991). This makes species of the *Salsola* genus ideally suited to invade agriculture systems (Young 1991). Species of the *Salsola* genus

are found in broad scale cropping and pastoral systems around the world, on every continent except Antarctica (Rilke 1999a).

*Effect in agricultural systems*

In Australia, *S. tragus* is a weed of the summer fallow period in the winter rain-fed grain cropping agricultural region (Mussell and Stewart 2004; Rilke 1999a). The summer fallow period extends from crop harvest in November and December to crop establishment in the following May and June, i.e. late spring, summer and autumn. Summers are arid in the mediterranean type climate of southern Australia, but *S. tragus* likely has the same deep root system and high water use efficiency evident in other, morphologically similar species of the *Salsola* genus (Dwyer and Wolde-Yohannis 1972; Fowler and Hageman 1978; Pan et al. 2001; Schillinger and Young 2000). It is likely that *S. tragus* removes a significant amount of soil moisture resulting from summer rainfall events and removes nutrients over the summer and autumn period. The effect of *S. tragus* on the yield of the following crop has not been investigated, although the removal of nitrogen and stored soil moisture from summer and autumn rainfall events by other summer weeds has been found to reduce the yield of the following crop in several regions of Australia, including the Western Australian wheat-belt (Osten et al. 2006). Removal of nitrogen by summer weeds can be remedied by application of nitrogen as fertiliser. However, rainfall is low in much of the Western Australian wheat-belt and crop yield in dry regions (i.e. regions with precipitation during the crop growing season less than 260 mm) is correlated to levels of stored soil moisture from the summer fallow period, as well as growing season rainfall (French and Schultz 1984; Tennant 2000). Stored plant available water per meter of soil from summer rains ranges from 40 mm in the poorest, sandy soils to over 200 mm in a sandy loam (Tennant 2000), if the water is not used by summer weeds.

*Salsola tragus* plants are not usually controlled consistently throughout the summer fallow, partly due to the difficulty in removing them. Herbicides are not very effective against plants past the seedling stage (Mussell and Stewart 2004), and cultivation is avoided in order to retain soil structure and avoid erosion (Turner 2004; Turner and Asseng 2005). Some growers attempt to remove summer weeds through repeated herbicide use against young plants, but most allow plants to grow during the summer fallow period and attempt to clear mature plants directly prior to the sowing operation.

However, time taken to clear plants can cause a delay in crop seeding, especially if the plants are not fully senesced. Any delay to crop sowing causes loss of moisture and reduced crop yield in Western Australia (Tennant 2000). The economic impact of *S. tragus* within cropping systems in Australia needs to be assessed, so that the extent and cost of weed management options that should be used against *S. tragus* can be determined.

*Salsola tragus* is likewise a weed of pasture systems in Western Australia, because sheep or cattle do not graze mature plants. Within pasture systems, *S. tragus* is a problem in Western Australia because herbicidal control options all impact on the desirable pasture species (Mussell and Stewart 2004). *Salsola tragus* is not commonly used as a forage plant in Western Australia, but internationally, several species of the *Salsola* genus have been considered as either useful forage species or toxic plants in pasture and rangeland environments. Species of the *Salsola* genus including *S. tragus* subsp. *iberica*, *S. paulsenii*, *S. orientalis* S.C. Gmel., *S. pestifer*, *S. rigida*, *S. richteri* (Moq.), *S. dendroides* Pall., *S. vermiculata* and *S. kali* have been considered as forage species, especially in arid, saline, degraded agricultural regions (Crompton and Bassett 1985; Fowler and Hageman 1979; Ghorbanian 2005; Ghorbanian et al. 2005; Hageman et al. 1978; Khassanov et al. 1994; Osman et al. 2006; Teimouri et al. 2005; Toderich et al. 2002). Hageman et al. (1978) investigated the suitability of *S. iberica* as a forage crop. The crude protein content of *S. iberica* was approximately 20% of dry plant weight for plants harvested before anthesis, and increased with nitrogen fertiliser. While protein content was reduced during and after flowering, protein content of the seeds was over 42% (Fowler and Hageman 1979). *Salsola iberica* could be harvested twice if the original harvest operation cut the plants above ground level (allowing plants to reshoot), although the protein content of the second harvest was reduced. *Salsola iberica* was compared to alfalfa (*Medicago sativa* L.), an alternative forage species. The *S. iberica* crop had 35% less protein and 27% less dry matter than the alfalfa crop, but was produced with half as much water (Hageman et al. 1978). In Australia, there is a significant amount of research conducted to identify and modify species that may be used as forage in degraded, saline regions (O'Connell et al. 2006; Rogers et al. 2005; Semple et al. 2006). While *S. tragus* in Australia is not particularly palatable to livestock, a selection program may identify ecotypes of *S. tragus* that are both palatable and tolerant of saline conditions. This species grows over summer and autumn, which is

usually a period of enforced fallow in Australian agricultural systems when little forage is available (due to low rainfall). Further, *S. tragus* can survive in marginal lands and may be of use in reclaiming saline or degraded areas of farmland as has occurred with other species of the *Salsola* genus (Hageman et al. 1978; Khassanov et al. 1994; Toderich et al. 2002).

Several species of the *Salsola* genus, including *S. tragus* in Western Australia, produce oxalates. These compounds can lead to oxalate poisoning in stock (Cannon et al. 1995; Jacob et al. 1992; Petheram and Kok 2003). *Salsola tragus* in W.A. has previously been claimed to cause oxalate poisoning (Petheram and Kok 2003), but most tests indicate that the oxalate concentration is not sufficient to poison sheep (Jacob and Peet 1989; Jacob et al. 1992). *Salsola pestifer* contained oxalates and nitrates in levels high enough to poison sheep in the presence of nitrogen fertiliser (Crompton and Bassett 1985). *Salsola tragus* plants may also produce high oxalate levels in the presence of nitrogen. In a pasture system with nitrogen fixing legume species, *S. tragus* may obtain sufficient nitrogen to produce oxalate in levels that are toxic to sheep.

*Legislative status*

*Salsola tragus* is not listed on the Noxious Weed List of any Australian State or Territory (Australian Weeds Committee 2007). Therefore, there is no legislation in place to regulate the control of this weed.

*Herbicide control*

Herbicide control is the most common management strategy applied to *S. tragus* in the Western Australian wheat-belt. Soil residual herbicides from the sulfonylurea chemical group are commonly used to control *S. tragus* during the period from spring to autumn, in the regions where *S. tragus* is the predominant summer weed. However, these herbicides cannot be used if legumes, canola or barley crops are planned for the following winter cropping season due to crop compatibility issues with herbicide residue (Hollaway et al. 2006; Mussell and Stewart 2004). Residual herbicides do not normally affect barley crops. However, in regions with low summer rainfall and alkaline soils, decay of residual herbicides is delayed to the extent where herbicide residues do affect barley crops (Hollaway et al. 2006). Unfortunately, barley is commonly the crop of choice in these alkaline regions where residual herbicides cannot be used, and it is

these regions where dense populations of *S. tragus* are most likely to be found (Mitchell and Wilcox 1988). Other herbicides used when sulfonylureas are not available include paraquat and diquat combined with 2, 4-D and triclopyr. However, plants that are not treated with either a systemic or residual herbicide will often rejuvenate from the base. In Canada, *Salsola pestifer* is successfully controlled with a variety of herbicides including those from the phenoxy, phenoxyalkanoic or phenoxyacetic acid chemical family, mixed with bromoxynil, dicamba or paraquat at the young, actively growing stage (Crompton and Bassett 1985). *Salsola iberica* is successfully controlled with various combinations of chlorsulfuron and glyphosate, paraquat, bromoxynil or metribuzin when herbicides are applied before or at anthesis (Young and Gealy 1986; Young and Whitesides 1987). Some of these herbicide combinations also reduce seed viability when sprayed during flowering. Herbicides applied directly to seeds in the laboratory were less effective than herbicides sprayed onto the plants in the field, where the plants could translocate herbicides into the seeds. Smaller seeds, possibly because they were smaller sinks of resources, had higher germination than large seeds after herbicide treatment (Young and Whitesides 1987).

*Herbicide resistance*

Sulfonylurea herbicides are the most effective herbicide choice for *S. tragus* in Australia, but evolved resistance to sulfonylurea herbicides in *S. pestifer* and *S. iberica* is a severe problem in America and Canada (Guttieri et al. 1992; Mallory-Smith et al. 1993; Morrison and Devine 1994). These herbicides provided a significant improvement to the control of these weeds when they were introduced in 1982, but resistance was recorded by 1989 (Thill et al. 1991). Mutations conferring resistance to ALS herbicides all appear to be nuclear-encoded, dominant or semi-dominant genes that are spread by both seed and pollen movement (Tranel and Wright 2002). As a result of the broad scale seed dispersal found in species of the *Salsola* genus, ALS resistance spread unusually quickly and was evident in 70% of the populations in Washington State after only 10 years of herbicide use (Mallory-Smith et al. 1993; Stallings et al. 1995). Saari et al. (1992) found moderate to high levels of resistance to sulfonylurea herbicides, including chlorsulfuron, imazapyr, sulfometuron methyl and triasulfuron in *S. iberica* populations. Resistance to chlorsulfuron was highest (0.5 g $ha^{-1}$ to achieve $GR_{50}$ for susceptible plants and >200 g for resistant plants) as this herbicide was most commonly used against *S. iberica* populations. Fitness of chlorsulfuron resistant

populations was equal to that of susceptible plants (Morrison and Devine 1994; Saari et al. 1994; Shaner 1991). At this stage, herbicide resistance has not been found in Australian populations of *S. tragus*. As sulfonylurea herbicides are one of the most effective control methods used against *S. tragus* in Australia, strategies should be employed to prevent the development of resistance to residual herbicides used for *S. tragus* control.

*Physical control*

Physical control of *S. tragus* is practiced in both cropping systems and pasture systems in Western Australia. Within a cropping system, cultivation may be used to kill plants before sowing the crop. *Salsola iberica* in the USA is effectively controlled by full cut cultivation, or rod weeders (blade cutters). These control methods kill mature plants by cutting the main stem free from the roots, and bury the seeds too deep for seedling establishment (Anderson et al. 1998; Blackshaw et al. 1994) (Figure 2.6). In a pasture phase in Western Australia, *S. tragus* can only be controlled physically, through heavy grazing while the plants are young or through crushing plants once they are mature, as herbicides negatively impact on desirable pasture species (i.e. broad leaf nitrogen fixing clover or medic species) (Mussell and Stewart 2004). In Canada, grazing controlled young *S. pestifer* plants (less than 6 cm tall) (Crompton and Bassett 1985). *Salsola vermiculata* plants in the USA died if they were cut at ground level, although if they were trimmed 1-3 cm above ground level they regrew and could not be distinguished from control plants 10 weeks later (Creager 1988). Research into optimal methods of physical control of *S. tragus* has not been conducted in Australia, but it will clearly depend on age of the targeted plants and their ability to regenerate.

Figure 2.6: A rod weeder (left) and blade-cutter (right) in Washington State, USA. The rod or blades are pulled through the soil, below the soil surface, to cut plants free from their roots.

*Biological control*

Biological control of *S. tragus* has not been attempted in Australia, but research outside Australia has identified several biological control agents of *S. tragus* or similar species of the *Salsola* genus. Some of these include fungi like *Uromyces salsolae* Reich. and *Colletotrichum gloeosporioides* Penz., moths such as *Coleophora klimeschiella* Toll and *C. parthenica* Meyerick, the gall midge *Desertovellum stackelbergi* Mamaev and the eriophyid mite *Aceria salsolae* de Lillo and Sobhian (Bruckart et al. 2004; Goeden and Pemberton 1995; Hasan et al. 2001; Muller et al. 1990; Smith 2005; Sobhian et al. 2003). In Australia, this weed probably does not have an economic impact large enough to justify the introduction of exotic biological control agents. However, the rust fungus *U. salsolae* is already found on *S. tragus* in Australia, as well as other species of the *Salsola* genus in the former USSR, Israel, Iran, Romania, France, Pakistan and Portugal (Hasan et al. 2001) (Figure 2.7). Trials in controlled conditions indicated that on *S. kali* populations, the rust fungus killed 50% of plants, prevented seed production on the surviving plants and significantly reduced plant size and weight. However, the fungus requires conditions of relatively low temperatures, low light availability and a dew period of at least four hours (optimal infection in 8-16 hrs) to successfully infect plants (Hasan et al. 2001). In Western Australian summers (i.e. the season of *S. tragus* growth), these conditions do not occur for a sufficient duration to allow for optimal infection, which would limit the spread of the fungus. *Uromyces salsolae* had no affect on other species in the Chenopodiaceae family, as well as species from five other plant families (Hasan et al. 2001). Research would be required to determine the suitability of biological control agents in Australia.

Figure 2.7: *Salsola tragus sensu lato* infected by the rust fungus, *Uromyces salsolae*, where the fungus is indicated by arrows (image from M. Jourdan, CSIRO Australia).

**Conclusions**

*Salsola tragus* is a weed of agricultural environments throughout Australia. The southwest Australian cropping system predominantly consists of winter cropping with an enforced summer fallow. Minimum tillage is used to conserve soil structure and soil moisture, leading to a heavy reliance on herbicidal weed control. Under this dry-land farming system, crop yield is usually related to moisture availability. *Salsola tragus* is a weed of the summer fallow period and is likely to have high water use efficiency, ensuring that it can remove all plant available moisture over summer and reduce the yield potential of the following crop. Herbicidal control of this weed is challenging, as the harsh environmental conditions reduce herbicide efficacy. Residual herbicides are the most effective control option for *S. tragus*, but resistance to residual herbicides develops easily in most species. Further, the efficient seed dispersal evident in *S. tragus sensu lato* allows herbicide resistance to spread quickly. *Salsola tragus* is currently of economic concern, but as a salt tolerant species this weed will become increasingly prevalent as salt affected land in the wheat-belt expands. Management of *S. tragus* needs to improve, but is hampered by the lack of information available on this species.

This review highlights the paucity of information on classification, biology, population ecology and management of Australian populations of *S. tragus*. There has been little research into the taxonomy of *S. tragus sensu lato*. As a result, there is considerable confusion regarding the classification, origin and status (native or exotic) of this species in Australia. There is virtually no information available on the biology, ecology and

impact of Australian populations of *S. tragus*. Research has been conducted on populations of *S. tragus sensu lato* found outside Australia, and other morphologically similar species of the *Salsola* genus, some of which is applicable to *S. tragus* in Australia. However, given that *S. tragus* has adapted to a very broad range of environmental conditions throughout the world, the characteristics of this species are likely to vary between continents. Research into the classification, biology, population ecology and impact of this species will probably lead to the development of effective management strategies for *S. tragus* in Australia.

From this review it was concluded that the issue of classification of the genus *Salsola* in Australia needed to be addressed, prior to research on biology and control of *S. tragus sensu lato*. The following two chapters address the issue of classification, through use of genetic and cytological analysis.

# Chapter 3

# Genetic analysis of the genus *Salsola* in southwest Australia[1]


**Abstract**

*Salsola tragus sensu lato* (Chenopodiaceae) is found throughout Western Australia and is considered to be an introduced weed in both natural and agricultural ecosystems. The taxonomic literature reports morphological variation within Australian populations of the weed, indicating that there were likely to be genetically distinct ecotypes or unidentified subspecies within the species. Seed was harvested from 26 geographically distinct populations of *S. tragus sensu lato* in southwest Australia and a molecular approach (RAMP) was used to detect genetic variation between populations. Out-groups used in this study included a population of *S. tragus* from Lind, Washington State, USA and *Maireana brevifolia* from Lake Grace, Western Australia. Four genetically distinct Groups were identified, which corresponded to observed morphological variations. Two of the identified Groups grew as weeds in the wheat-belt of Western Australia. A third Group was found in the rangelands to the north-east of the wheat-belt. The fourth Group was restricted to littoral habitats along the coast. The Groups identified were distantly related to the out-groups (approximately 60% similarity). The predominant wheat-belt weed (Group A), which was previously classified as *S. tragus* subsp. *tragus*, was identified as *S. australis*. Plants from the other three Groups remain unclassified. Further research is required to positively identify these Groups and determine the origin of each. An analysis of *Salsola* across Australia is needed to establish if there are further genetically distinct groups, and to determine if *Salsola* taxa are native to Australia and not introduced as is usually assumed.


## Introduction

*Salsola tragus sensu lato* is a highly invasive agricultural and environmental weed, surviving in a broad range of environments in Australia, North and South America,

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Africa, Europe and Asia (Mitchell and Wilcox 1988; Rilke 1999a). The ability of a plant species to become an invasive weed is partially attributed to the possession of genetic traits that allow for survival in variable environments, but it is likely that genetic adaptation to conditions in the new environment also plays a significant role (Baker 1965; Clements et al. 2004; Neuhauser et al. 2003; Sakai et al. 2001). Invasive weeds experience strong selection pressure created by control efforts and the constant changes to the environment or agricultural system they inhabit. Such selection pressure can result in the rapid evolution (over years or decades) of ecotypes that are adapted to the new conditions (Neuhauser et al. 2003; Thompson 1999). Several species of the *Salsola* genus have genetic traits that allows them to survive and successfully invade ruderal habitats (Mitchell and Wilcox 1988; Rilke 1999a; Young 1991). However, given their success in invading such a broad range of natural and agriculture environments, it is also likely that they rapidly evolve ecotypes genetically adapted to new environments.

*Salsola tragus* has existed in Australian agricultural systems for over 200 years and has invaded very diverse habitats (Beadle 1981; Brown 1810; Smith et al. 1980). There are established populations in rainfed agricultural environments, high and low rainfall woodlands, shrub lands, grasslands, sandy sites, semi-deserts and coastal habitats (Beadle 1981; Rilke 1999a; Smith et al. 1980). Despite this wide habitat range, *Salsola tragus* is the only recognised species of the *Salsola* genus in Australia (Rilke 1999a; Wilson 1984). Within Australia there are three recognised subspecies of *S. tragus*. *Salsola tragus* subsp. *tragus* is found throughout Australia and is a common agricultural weed. *Salsola tragus* subsp. *grandiflora* occurs within the subtropical northern regions and is unique to Australia. *Salsola tragus* subsp. *pontica* exists in littoral zones on the north-east and south-west coast of Australia (Rilke 1999a). There appear to be a very diverse range of morphologically distinct ecotypes included within this species as a whole, and especially evident within *S. tragus* subsp. *tragus* (Rilke 1999a).

Descriptions of this species in Australia acknowledge the morphological diversity evident within *S. tragus* populations, which is significantly greater than the polymorphism evident within *S. tragus* populations found on other continents, but attribute the differences to mutations or environmental conditions and do not attempt to further subdivide the species (Rilke 1999a; Wilson 1984). However, within the *Salsola* genus as a whole, the assignation of scientific names to various species has a confusing

history (Rilke 1999b; Young 1991). In fact, until recently, *S. tragus* has been used almost interchangeably with *S. kali*, *S. pestifer* and *S. iberica* (Beatley 1973; Rilke 1999a). In Australia, populations of *Salsola* were originally classified as *S. kali* (Smith et al. 1980), and then as *S. australis* and *S. macrophylla* (Brown 1810). In Flora of Australia, Australian material was reclassified as *S. kali* and a recent international review of the genus *Salsola* section *Salsola sensu lato* conducted by Rilke reclassified Australian populations as *S. tragus* (Rilke 1999a; Wilson 1984). For the purpose of this work, the classification system developed by Rilke (1999a) has been accepted. Given the traditional confusion in classification of species within this genus, it is possible that other species of the *Salsola* genus are found in Australia. Alternatively, if *S. tragus* is the only species of the *Salsola* genus in Australia, it occupies such a broad range of environments and shows such a high degree of morphological diversity that it is possible that genetically distinct ecotypes, or even separate species or subspecies, have evolved in Australia.

To determine the genetic structure of *S. tragus* populations in Western Australia, we employed the RAMP (random amplified microsatellite polymorphism) method. This method has been used in other studies to determine the genetic relationships of plant species, from species to cultivar level (Becker and Heun 1995; Cheng et al. 2001; Davila et al. 1999; Richardson et al. 1995; Sanchez de la Hoz et al. 1996; Wei et al. 2005; Wu et al. 1994; Yuan et al. 2005). A RAMP assay combines microsatellites and RAPD markers to detect polymorphisms. The advantages of this method are that it is reliable, fast, relatively inexpensive, detects a high level of variability and does not require *a priori* DNA sequence information (Richardson et al. 1995; Wu et al. 1994). This method was employed because we did not know how closely related Western Australian populations of *S. tragus* are, and therefore what level of variability would be needed to distinguish between them. This work tested the hypothesis that there are genetically distinct populations of *S. tragus* in southwest Australia.

**Methods**

*Plant material*

*Salsola tragus* plants were sampled from 26 sites in southwest Australia during March and April 2005 (Figure 3.1). At each site seeds were harvested from 15 randomly selected plants. Site locations were recorded using GPS (WGS84) and at least one

voucher specimen was taken from each population where plants were still alive. Voucher specimens have been lodged at the Western Australian Herbarium (PERTH) and listed in Appendix A.

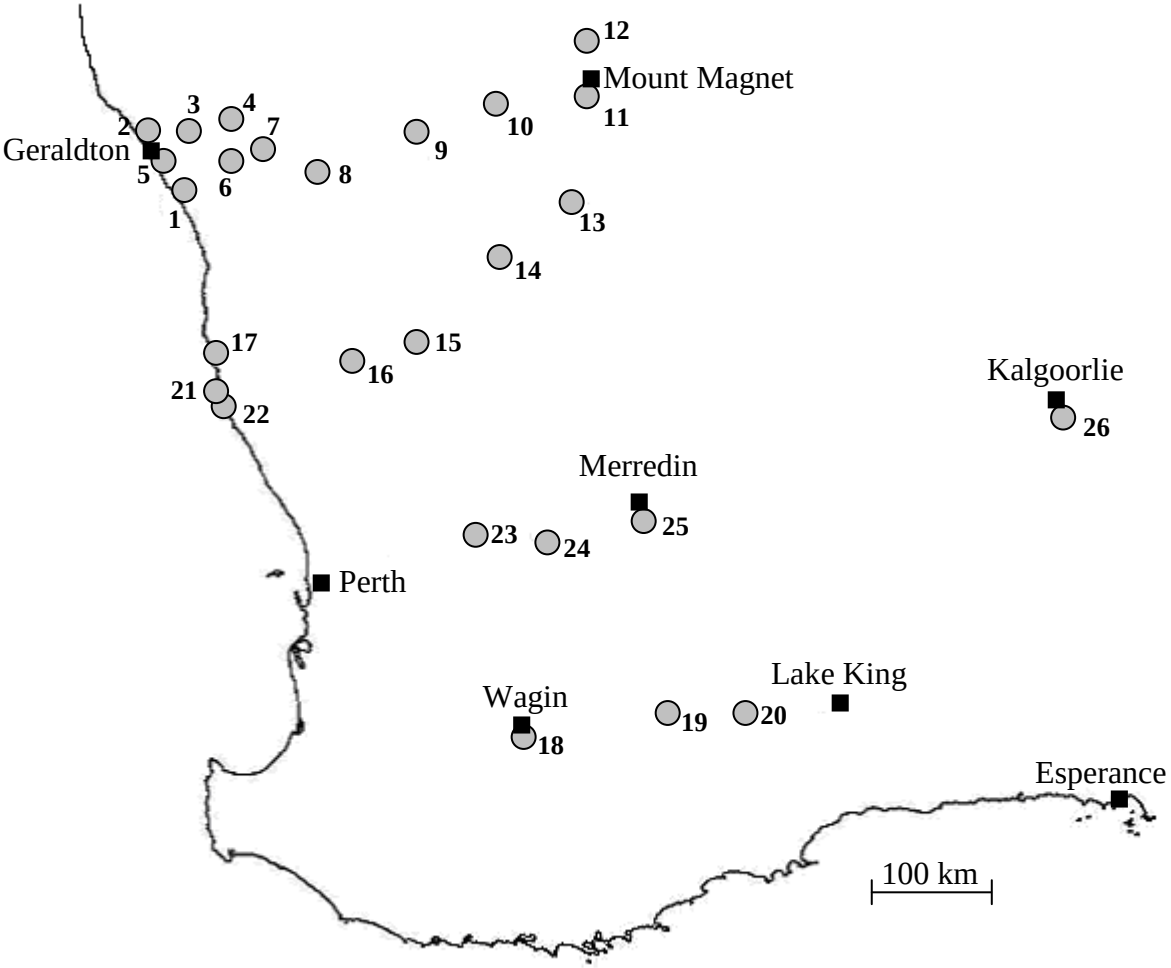


Figure 3.1: *Salsola tragus sensu lato* collection sites in Western Australia, where circles indicate collection sites and squares indicate the location of major cities/towns.

Seed samples from five plants per site were selected and 200 seeds from each sample were sown in pots (116 mm diameter, 117 mm tall) containing potting mix (50% mulched pine bark, 25% sand 35% peat moss), at a depth of 1 cm. Seed viability varied from less than 1% to 85%. Sites 3, 8, 16 and 19 had very poor seed viability, and were not used in the following analysis. When seedlings reached the four leaf stage, leaves were harvested from five seedlings per pot and bulked to acquire 0.1 g of leaf material, directly before DNA isolation commenced. The out-groups used in the analysis were *S. tragus*, from Lind, Washington State USA (S 47$^{o}$00.014$^{'}$ E 118$^{o}$20.236$^{'}$) and *Maireana brevifolia* (R.Br.) Paul G. Wilson (Chenopodiaceae) from Lake Grace, Western Australia (S 33$^{o}$07.384$^{'}$ E 118$^{o}$28.568$^{'}$). The *S. tragus* seeds from Washington State

were germinated on damp filter paper in a PC2 facility, and leaf material was harvested as above. Branches from *M. brevifolia* plants were harvested in April 2005 and stored at -80$^{o}$C.

*RAMP procedure*

The DNA was isolated from 93 samples using the QIAGEN DNeasy® Plant Mini DNA isolation kit (QIAGEN 2004). This method produced 100 µL DNA solutions, which were stored at -20$^{o}$C. A PCR solution was prepared by incubating 10.5 µL sterile water, 9 µL 5 x kinase buffer, 1.5 µL T4 polynucleotide kinase, 9 µL of γ-33P ATP and 30 ng µL$^{-1}$ of SSR primer stock (Table 3.1) in a thermocycler (Hybaid OmniGene) at 37$^{o}$C for 1 hour and then 70$^{o}$C for 10 min, before adding 45 µL of this solution to 50 µL of RAPD primer stock (Table 3.1) and 800 µL of PCR mix. A labelling stock was prepared by mixing 2.1 µL sterile water, 1.8 µL 5 x kinase buffer, 0.3 µL T4 polynucleotide kinase and 1.8 µL of γ-33P ATP with 3 µL ladder stock and 180 µL TBE buffer and incubating as above. The concentration of each DNA sample was adjusted to between 5 and 25 ng µL$^{-1}$ by diluting with sterile water (Water for Injections BP, Astra Pharmaceuticals Pty. Ltd.). The DNA amplification was carried out by incubating solutions containing 1.5 µL of a DNA sample and 8.5 µL of the PCR solution in the thermocycler, using the PCR cycles described by Wu *et al*. (1994). The PCR products were resolved on a polyacrylamide denaturing sequencing gel and the amplified marker bands were detected by autoradiography according to the method described by Yuan (2005).

*Data analysis*

Polymorphic RAMP markers were scored as 1 for present and 0 for absent, to construct a binary dataset. A regression analysis ($r^2 = 1$) to compare the size of the ladder bands and the distance they moved was used to determine the size of the RAMP markers. Morphological differences between plants at the 26 sites allowed populations to be separated into four Groups (A, B, C and D, Figure 3.2). Each sample was allocated into a Group and a simple matching similarity matrix was calculated and used to produce an MDS (non-metric multi-dimensional scaling) analysis (with 50 restarts and a stress of 0.16 for the best two dimensional configuration). The data were subjected to a one-way ANOSIM (analysis of similarity), with 9999 permutations. A dendrogram based on group averages was constructed, and a SIMPROF (similarity profile) test with 1000

permutations at a significance level of 1% was conducted on the dendrogram (PRIMER 6.1.6) (Clarke and Gorley 2006). A distance matrix based on total character difference was calculated based on the original dataset and UPGMA (unweighted pair-group method with arithmetic averages) and Neighbour-Joining were adopted to construct dendrograms. Bootstrap analysis with UPGMA search was based on 2000 replications (PAUP) (Swofford 2001).

**Results**

The RAMP analysis generated sufficient genetic polymorphisms for the analysis of these *Salsola* collections. Each of the eight primer combinations used in the RAMP analysis produced from 5 to 90 polymorphic bands. In total, 422 polymorphic bands were identified (Table 3.1), ranging in size from 120 to 2873 bases. A section of the amplified products for selected samples, from the primer combination MF 52 and OPC 18, is shown in Figure 3.2.

Table 3.1: The combinations of SSR and RAPD primers that produced polymorphic bands in DNA samples from *Salsola tragus sensu lato*.

| SSR primer | Sequence (5'-3') | RAPD primer | Sequence (5'-3') | Number of polymorphic bands |
|---|---|---|---|---|
| MF 11 | GGAC(CT)6 | OPC 03 | GGGGGTCTTT | 5 |
| MF 43 | CCTC(AAG)5 | OPC 04 | CCGCATCTAC | 46 |
| MF 43 | CCTC(AAG)5 | OPC 08 | TGGACCGGTG | 74 |
| MF 52 | GGG(AAG)4 | OPC 01 | TTCGAGCCAG | 77 |
| MF 52 | GGG(AAG)4 | OPC 18 | TGAGTGGGTG | 90 |
| MF 153 | CCTT(AC)6 | OPC 20 | ACTTCGCCAC | 50 |
| MF 155 | CAAC(TG)6 | OPC 07 | GTCCCGACGA | 58 |
| MF 155 | CAAC(TG)6 | OPC 09 | CTCACCGTCC | 22 |

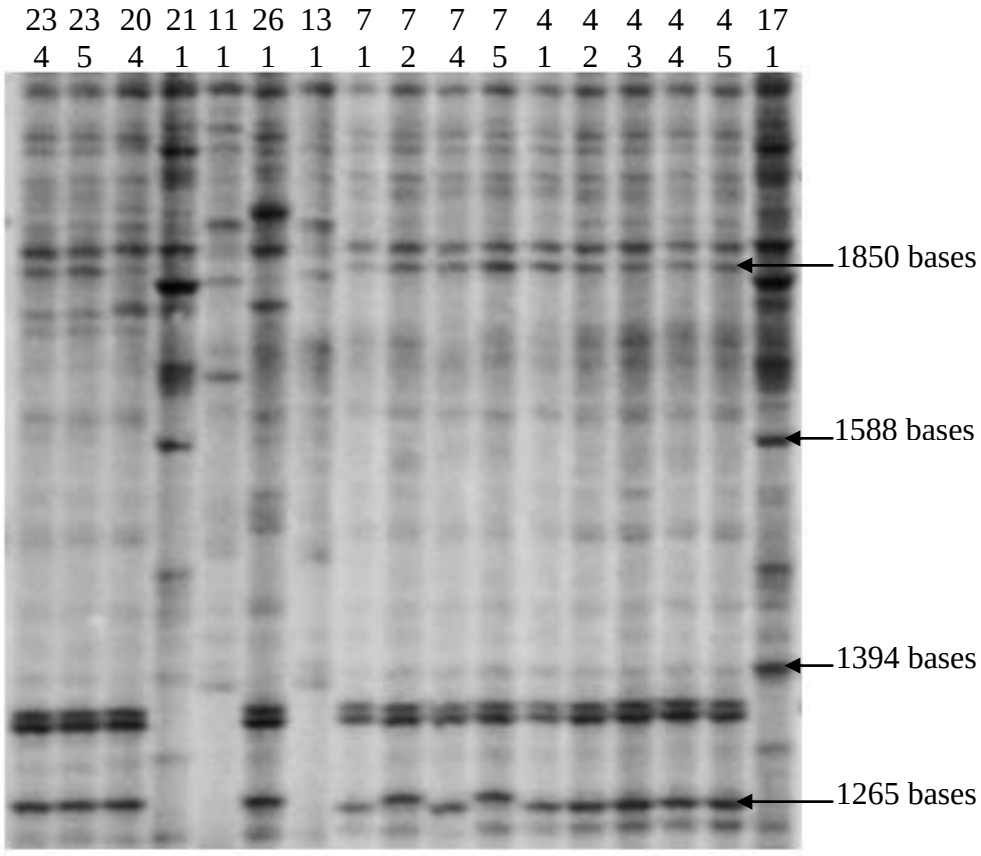


Figure 3.2: Section of a RAMP amplification profile obtained with the primer combination MF 52/OPC 18, for selected DNA samples. The lanes are labelled with collection site number for each plant population (top row) and the number assigned to individual plants at each site (bottom row). The size, in bases, of selected polymorphic bands is indicated by the arrows.

Twenty three group specific markers were identified from seven of the primer combinations (Table 3.2). The primer combination MF 153 OPC 20 provided specific markers for Group B, C and D and the combination MF 155 OPC 07 provided specific markers for Group A, B and C. None of the primer combinations produce specific markers for all four Groups.

Table 3.2: Specific markers for Groups of the *Salsola* genus in south Western Australia generated by RAMP. Each marker is indicated by the primer combination which generated it and its size (in base pairs).

| Group | Number of specific markers | Marker details |
|---|---|---|
| A | 8 | MF 52 OPC 01, 1152; MF 52 OPC 18, 725; MF 155 OPC 07, 1896; MF 155 OPC 07, 1431; MF 155 OPC 07, 845; MF 155 OPC 07, 830; MF 155 OPC 07, 526; MF 155 OPC 07, 517 |
| B | 5 | MF 43 OPC 08, 868; MF 153 OPC 20, 1299; MF 155 OPC 07, 2181; MF 155 OPC 07, 1865; MF 43 OPC 04, 1210 |
| C | 6 | MF 52 OPC 18, 440; MF 153 OPC 20, 369; MF 155 OPC 07, 120; MF 43 OPC 04, 2253; MF 43 OPC 04, 1299; MF 155 OPC 09, 1601 |
| D | 4 | MF 52 OPC 01, 2308; MF 52 OPC 01, 409; MF 52 OPC 18, 1117; MF 153 OPC 20, 2146 |

The MDS, ANOSIM and dendrogram cluster analyses based on RAMP data unequivocally clustered the 26 populations into four genetically distinct groups, which corresponded to the four groups referred to as Group A, B, C and D (Figure 3.3, 3.4, 3.5). Plants were grouped according to morphological differences readily apparent in the field. Group A was distributed over most of the wheat-belt, at sites 1, 4, 7, 15, 20, 23, 24, 25 and 26 (Figure 3.1). These plants were characterised by single seeds (within the fruiting perianth) at the base of the leaves on each branch (Figure 3.3). Group B existed in the rangelands found to the north-east of the wheat-belt, at sites 9, 10, 11, 12, 13 and 14. These plants were distinguished from Group A plants by the fruiting structures. Fruit formed at the base of each leaf, but congested fruits on globular spikes attached to branches were also apparent. Group C was found at two isolated locations in the wheat-belt, sites 6 and 18. These plants had all fruiting structures on globular spikes attached to the main branches. Plants of Group A, B and C appeared to be annual plants. Following senescence these plants detached from their root systems to become mobile, as usual for *S. tragus* plants (Mitchell and Wilcox 1988). Group D was restricted to sand dune systems along the coast on the north-west side of the wheat-belt, at sites 2, 5, 17,

21 and 22. These plants were darker green, with flowers attached to the main branches and shorter, more succulent bracts and leaves. They appeared to be perennials (i.e. individual plants continued vegetative growth and reproduction for more than a single year, possibly several years) and showed no sign of becoming mobile at senescence.


Figure 3.3: Flowers and fruits of *Salsola tragus sensu lato* groups in south Western Australia, labelled Group A, B, C and D.

The MDS and ANOSIM analysis indicated that the samples clustered strongly according to morphological Groups (Figure 3.4 and Table 3.3; R: 0.967, P: 0.01). There was slightly more variation between samples classified as Group C than between samples within Group A, B and D, but there were also few samples within Group C. Group B and D had slightly more variation within the groups than Group A. However, most of this variation can be accounted for by the outlier in each of these populations (site 2, plant 5 in Group D and site 12, plant 2 in Group B).

Table 3.3: Global R statistic and pair-wise comparisons from ANOSIM performed on the samples divided into the four genetic and morphological Groups (Group A, B, C and D). The number of permutations was 9999 and the significance level for each test was 0.01. ANOSIM was based on a simple matching similarity matrix.

| Groups | R statistic |
|---|---|
| Global test | 0.983 |
| A, C | 0.999 |
| A, D | 0.992 |
| A, B | 0.997 |
| C, D | 0.896 |
| C, B | 0.979 |
| D, B | 0.941 |

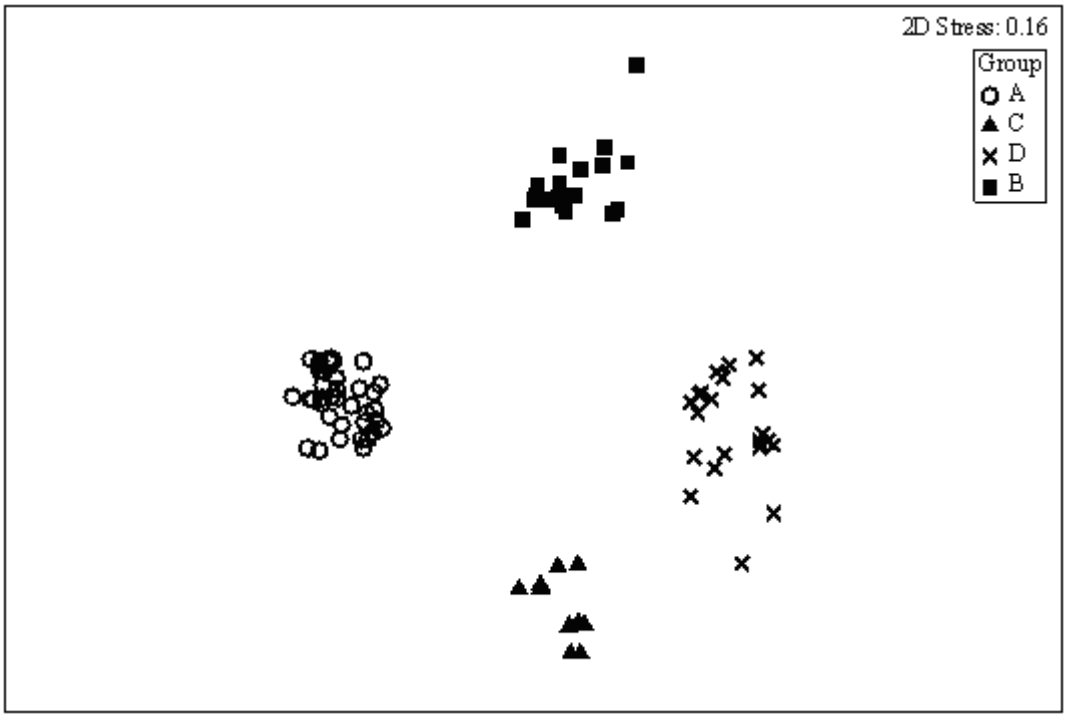


Figure 3.4: Two-dimensional image of the MDS analysis of DNA data divided the collected *Salsola* genotypes into four distinct groups matching the morphological groups as illustrated in Figure 3.3. MDS analysis was based on a simple matching similarity matrix.

The group average dendrogram confirmed that the samples could be separated into the four Groups and showed that Group A, B and C were more closely related to each other than to Group D (Figure 3.5). The four Groups were approximately 71% similar. The UPGMA and Neighbour-Joining dendrograms showed almost identical relationships between samples in the four Groups, and so were not included. The bootstrap analysis indicated that grouping of samples into genetically distinct Groups was strongly supported. Group A, B or D were grouped with 96-100% bootstrap support and site 6 and 18, which form Group C, grouped with 84% support. The SIMPROF analysis confirmed that the relationships between the populations within a Group and between the four Groups were significant. All four Groups were approximately 60% similar to the out-groups *S. tragus* and *M. brevifolia*, with 100% bootstrap support (Figure 3.5). The samples from a single site were usually more closely related to each other than to samples from other sites, a grouping supported by both the bootstrap and SIMPROF analysis.

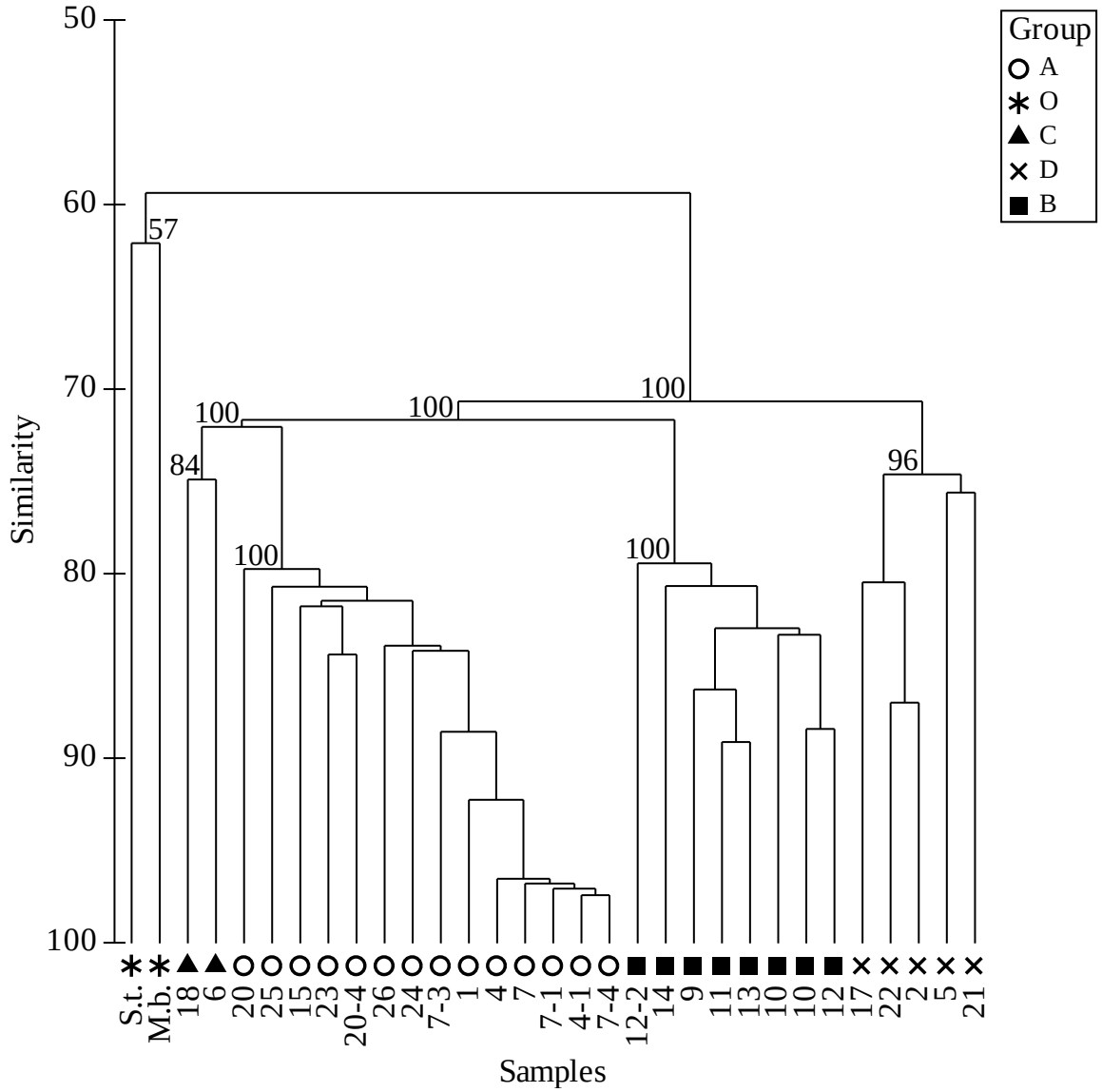


Figure 3.5: A group average dendrogram constructed from the 93 samples. Grouping structure of plants from the same site was usually not significant (according to the SIMPROF analysis), and so the branches were collapsed, leaving 31 groups showing. Labels indicate the collection site number for collapsed branches (i.e. 18 represents a branch containing plant samples 1-5 from site 18), or the site and plant number for branches showing single plant samples (i.e. 20-4 is plant sample 4 from site 20). The out-groups, *Salsola tragus* and *Maireana brevifolia*, are labelled as S.t. and M.b. The symbols indicate the four genetic/morphological groups and the out-groups, as indicated by the key. The axis to the left indicates the similarities between individuals, calculated from a simple matching coefficients matrix. The numbers within the dendrogram at branching points indicate the bootstrap values, based on 2000 reiterations.

**Discussion**

Based on the RAMP data, populations of the *Salsola* genus in southwest Australia could be clustered into four genetically distinct Groups, which have their own morphological features and are each restricted to specific geographic locations. The dendrogram indicated that Group A, B and C were genetically more similar to each other than to Group D. Groups A, B and C were also morphologically more similar to each other than to Group D, since they were annual plants that detached from their root systems to become mobile following senescence and Group D were perennial plants that did not detach from their roots. All Groups were genetically distinct from *S. tragus*. This work confirmed the hypothesis that there are genetically distinct groups of *Salsola* within Western Australia.

It was suggested (Introduction) that the rapid evolution necessary to develop such a high level of polymorphism in *S. tragus* over the 200-300 years it has been in Australia may have been possible due to the strong and directional selection pressure resulting from exposure to variable environments and agricultural systems (Clements et al. 2004; Neuhauser et al. 2003; Rilke 1999a). However, it was assumed that the morphological variation seen between ecotypes was the result of simple mutations and the majority of the variation was due to environmental rather than genetic variation (Rilke 1999a; Wilson 1984). It is now evident that there are genetically distinct Groups rather than ecotypes present in Western Australia, and it is likely that more than 200 years of evolution was necessary to achieve this level of segregation between them. This raises the possibility that some or all of these Groups are native and have been evolving in Australia for a considerably longer time span than 200-300 years.

The RAMP analysis proved to be a very sound technique for identifying genetic variation. A large number of marker bands were identified, which were unambiguous and easy to score on the autoradiographs. The resulting multivariate analysis was highly robust. The Group specific markers identified can be used to distinguish between these Groups in future. Taxonomists have struggled to correctly classify species within the *Salsola* genus, as evidenced from the work of Wilson (1984) and Rilke (1999a). In particular, *S. tragus* is morphologically highly similar to Group A, even though they are clearly genetically distinct. This molecular genetic technique has proved to be a

valuable tool in identifying polymorphisms within the *Salsola* genus and can be used as a basis for future research into the systematics of the genus.

Three *Salsola* species analysed by Ryan and Ayres (2000) were approximately 45 to 60% similar, based on a simple matching coefficients matrix. Species within the *Beta* genus, a close relative to the *Salsola* genus, show 40-60% similarity between species (Shen et al. 1996). Given that the four Groups identified here were 60% similar to *S. tragus* (and *M. brevifolia*), it is likely that they are not *S. tragus*. They are 71% similar to each other and so are unlikely to be separate species, but may be separate subspecies. Further research by plant taxonomists is required to classify these Groups. Previously it was assumed that species of the *Salsola* genus show pronounced intra-specific and limited inter-specific variation (Rilke 1999a; 1999b; Wilson 1984). However, this study has found the reverse; there was pronounced inter-specific and limited intra-specific variation. There are other populations throughout Australia that are currently classified as *S. tragus*, but are morphologically distinct from either *S. tragus* or the Groups identified here (Rilke 1999a), suggesting the possibility that there are other unidentified taxa of the *Salsola* genus in Australia.

Group A (currently classified as *S. tragus* subsp. *tragus*) corresponds to the species *S. australis*. The botanist Robert Brown described two species of the *Salsola* genus from specimens collected in Australia during 1802-1805. One of these was *S. australis*, a fleshy, much-branched herb, the voucher specimen of which was collected from Petrel Bay, Isle St. Francis, South Australia (S 32$^{o}$30.11$^{'}$ E 133$^{o}$17.41$^{'}$) (Brown 1810). The location from which this *S. australis* voucher specimen was collected was a littoral habitat and in the current study Group D was found in littoral habitats rather than Group A. However, from our surveys, Group D is restricted to northern coastal areas and was not found south of Cervantes (approximately 150 km N of Perth) in Western Australia. Group A was only found in the wheat-belt in this study, but samples of this morphological Group within the Western Australian herbarium have previously been found on the coast (i.e. PERTH 05347718, PERTH 05249368, PERTH 06633498). Rilke (1999a) visually examined the original voucher specimen of *S. australis* (The Natural History Museum, London Department of Botany, 000016765, BM) collected by Brown and concluded that the typical, southern inland form of *Salsola* throughout Australia (Group A) was the same as *S. australis*, but concluded that *S. australis* did not

morphologically vary from *S. tragus*. However, work done by Ryan and Ayres (2000) indicated that within California there were three genetically distinct *Salsola* species, *S. tragus*, *S. paulsenii* and an unidentified species that was morphologically similar to *S. tragus*. Unpublished work by Dr Fred Hrusa (Californian Department of Food and Agriculture Herbarium) has indicated that the unclassified species identified by Ryan and Ayres (2000) and Group A identified here are the same species, and his comparison of these species with the voucher specimen of *S. australis* confirmed that both are *S. australis*. *Salsola australis* is an introduced species in the USA and may be native to Australia.

The other three Groups (B, C and D) remain unclassified. Neither Group matches the description of *S. tragus* subsp. *pontica* or subsp. *grandiflora*, although this survey did not cover the northern reaches of Western Australia where *S. tragus* subsp. *grandiflora* is found (Rilke 1999a). Group D matches Robert Brown's description of *S. macrophylla* as an erect, woody plant (Brown 1810). However, the original voucher specimen cited by Brown was collected in Queensland (Thirsty Sound, S 22°08.00' E 150°51.00') (1810) and was not available for comparison to the Western Australian material. Rilke (1999a) states that *S. macrophylla* is not a separate species. She concludes that plants previously classified as *S. macrophylla* are actually *S. tragus* plants that have unusually succulent leaves and bracts (diameter >2 mm) as a result of growing in highly saline environments. Group B and C are more similar to Group A than to Group D and it is likely they are subspecies of *S. australis*. Both Groups match the description of *S. kali* var. *strobilifera* by Wilson (1984), which has previously been applied to specimens with fruits congested on globular or ovoid spikes. However, it was previously unclear if these plants were a distinct taxon or a mutation arising in several species within the *Salsola* genus (Rilke 1999a; Wilson 1984).

The origin of the *Salsola* genus in Australia remains unclear. The number of distinct Groups in Western Australia and the close relationship between them indicates that at least some of these Groups are likely to be native, rather than introduced species. The historical evidence indicates that species of the *Salsola* genus were established in Australia prior to European invasion (Brown 1810; Smith et al. 1980), and were recorded in all states of Australia during the 19th century (Domin 1921; Rilke 1999a). This may indicate that species of the *Salsola* genus were native throughout Australia.

Alternatively, it may be that these species were native to the eastern states of Australia, and their range expanded with the expansion of agriculture throughout the continent. While *S. australis* was recorded in South Australia in 1802 as a native plant by Brown, populations morphologically similar to *S. australis* have also been described in South Africa, although the first specimen was not recorded until 1885, significantly later than in Australia (Brown 1810; Rilke 1999a). However, Australia had several well established weeds prior to the 1800's (Groves and Burdon 1986; Michael 1972). Robert Brown noted 29 weed species in Australia during 1802 and 1804, many of which were present in South Australia (Groves and Burdon 1986; Kloot 1985). Further, *S. australis* (1810) and *S. kali* (1980) were found in littoral habitats, and there are many coastal species that are likely to have arrived in Australia prior to European invasion through long distance seed dispersal (Bean 2007; Groves and Burdon 1986; Kloot 1984).

The widespread sampling of *Salsola* plants in southwest Australia leads us to believe that *S. tragus* is not present, which has several significant implications for weed management. Firstly, *S. tragus* should be assessed for its quarantine risk status and then excluded from Western Australia in the future to protect the State from this highly invasive weed. Secondly, if future research concludes that these species are native to Western Australia rather than exotic invasive species then the *Salsola* species found in natural ecosystems do not need to be eradicated or controlled. However, even if native, *S. australis* is a successful agricultural weed requiring management. *Salsola australis* (referred to as *S. tragus* subsp. *tragus*) was recorded as an agricultural weed throughout Australia by 1921, and is also an introduced weed in the USA (Bentham 1870; Domin 1921; Rilke 1999a; Ryan and Ayres 2000).

This research shows that there are four distinct groups from the genus *Salsola* in southwest Australia, none of which are the species *S. tragus* subsp. *tragus* recognised in North America. One Group has been positively identified as *S. australis*, but the other three Groups cannot be matched to recognised species, and the origins of all four Groups are ambiguous. The literature indicates that there is a large degree of polymorphism evident in this genus throughout Australia, which implies that similar studies in other States would reveal other species of the *Salsola* genus. This research needs to be extended to include northern and eastern Australian material in a genetic analysis and to ascertain ploidy levels of Australian taxa. Possibly genetic analysis of

the *Salsola* genus worldwide needs to be conducted to determine the status of Australian taxa. The morphology of the *Salsola* genus needs to be re-examined and complemented with taxonomic work to provide names for the unidentified Australian material. A study of the associated pathogens and fauna might help identify which Groups are native, and provide potential biological control agents for use in North America where *S. australis* is an invasive weed (Ryan and Ayres 2000) (Dr Fred Hrusa, pers. comm.).

# Chapter 4

# Chromosome number of *Salsola* species in southwest Australia

**Abstract**

Chromosome number was investigated in plants of the *Salsola* genus found within southwest Australia, to facilitate the identification of these significant agricultural weeds. Ploidy levels were examined in 283 seedlings from seed samples that were harvested from plants at 19 sites. Seedlings from populations of *S. australis* and *S. tragus* obtained from the USA were included for purposes of comparison. All Australian populations of *Salsola* were diploid 2*n* = 18, as was *S. australis* from the USA. *Salsola tragus* was confirmed as tetraploid 2*n* = 36. This supports the view that the four variants found in Western Australia, which were previously classified as *S. tragus*, are either *S. australis*, or close relatives of this species.

## Introduction

Accurate knowledge of chromosome number in weed species is important for reasons such as species identification, to assess the variation within a species or genus, and as evidence to support indications of the origin of a species, all of which may assist in development of weed management strategies (Clements et al. 2004; Neuhauser et al. 2003; Sakai et al. 2001). There are several invasive weed species of natural and agricultural environments found within the genus *Salsola* (Rilke 1999a). Accurate identification of species within this genus is challenging, due to the high levels of intra-specific and low levels of inter-specific morphological diversity (Rilke 1999a; 1999b). This confusion in identification has previously hindered the development of control measures for this weed (Bruckart et al. 2004; Ryan and Ayres 2000; Smith 2005). Since traditional morphological analysis cannot be used to accurately identify species of the genus *Salsola*, both genetic and cytological methods have been employed (Ryan and Ayres 2000).

Cytological analysis of species of the *Salsola* genus indicate a base chromosome number of $x = 9$, with polyploidy evident in both weed and non-weed species. Diploid, tetraploid and hexaploid forms are reported both from the native range of the *Salsola*

genus in Europe and Asia, and the introduced range of North America and Canada (Table 4.1).

Table 4.1: Previously published chromosome counts for species of the genus *Salsola*.

| Taxon | Location of samples in study | Chromosome number | Reference |
|---|---|---|---|
| *S. australis* R. Br. | USA, California | 2*n* = 18 | Ryan and Ayres (2000), Fred Hrusa (2006, pers. comm.) |
| *S. collina* Pall. | USA (introduced range) | 2*n* = 18 | Pohl and Gillespie (1959), Mosyakin (1996) |
| *S. lanata* Pall. | Central Asia | 2*n* = 18 | Wojnicka-Poltorak et al. (2000) |
| *S. praecox* Litv. | Central Asia | 2*n* = 18 | Wojnicka-Poltorak et al. (2000) |
| *S. soda* L. | Asia | 2*n* = 18 | Zakhar'yeva (1985) |
| *S. kali* L. | Canada, Summerville | 2*n* = 36 | Löve (1970) |
| *S. paulsenii* Litv. | Europe and Asia | 2*n* = 36 | Mosyakin (1996) |
| *S. pestifer* A. Nels. | Canada, Brandon | 2*n* = 36 | Crompton and Bassett (1985; 1970), Mosyakin (1996) |
| *S. tragus* L. | Canada | 2*n* = 36 | Mulligan (1961), Löve (1970) |
| *S. paulsenii "*lax form"* | North America | 2*n* = 54 | Arnold (1972) |

*Taxon identification uncertain and maybe of hybrid origin (http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=200006926).

Species of the *Salsola* genus in Australia are minor weeds throughout the entire continent, and economically significant weeds in the northern and southern regions of the wheat-belt of Western Australia (Mitchell and Wilcox 1988; Mussell and Stewart 2004; Wilson 1984). Management of these weeds would likely be assisted by accurate

species identification, but this has proven challenging. The *Salsola* genus in Australia has a confusing taxonomic history (Australian Plant Name Index 2005). Species were originally classified as *S. kali* by Banks in 1770 (Smith et al. 1980), as *S. australis* and *S. macrophylla* by Brown (1810), as *S. kali* by Wilson (1984) and finally as *S. tragus* by Rilke (1999a). Currently, *Salsola tragus* subsp. *tragus* and subsp. *grandiflora* is the only recognised species of the *Salsola* genus within Western Australia (Rilke 1999a; Western Australian Herbarium 2007).

However, the classification of these species has again been questioned. In Chapter 3 it was shown that the genus *Salsola* in southwest Australia has four genetically distinct variants, none of which were closely related to *S. tragus*. The predominant variant, a ubiquitous agronomic weed, was previously classified as *S. tragus* subsp. *tragus*. Comparison of this weed species with the *S. australis* lectotype (000016765, BM) collected by Robert Brown (1810) suggested that it could be reclassified as *S. australis* (Dr Fred Hrusa, Californian Department of Food and Agriculture Herbarium, pers. comm.). The remaining variants (labelled Group B, C and D) remain unidentified. They are genetically more similar to *S. australis* than to *S. tragus*, although morphologically they are distinct from either species.

The ploidy level of the Australian material has not been examined. Given that *S. tragus* has $2n = 36$ chromosomes and *S. australis* has $2n = 18$ chromosomes (Dr Fred Hrusa, pers. comm.) (Löve 1970; Mulligan 1961; Ryan and Ayres 2000), assessing somatic chromosome numbers would confirm that the incorrectly named *S. tragus* subsp. *tragus* in Western Australia is *S. australis*, and offers further evidence that the remaining three genetically distinct groups are more closely related to *S. australis* than to *S. tragus*.

**Materials and Methods**

Seeds were collected at 19 sites throughout southwest Australia. The GPS coordinate for each site is shown in Table 4.2. Voucher specimens from each site have been lodged at the Western Australian Herbarium (PERTH) and are listed in Appendix A. Seeds were collected from representative populations of the four genetically distinct putative taxa identified in a previous genetic analysis (Chapter 3), labelled *S. australis*, Group B, C and D. Populations of *S. tragus* and *S. australis* from Davis and Santa Nella, California USA, were included. These populations had previously been classified using

genetic analysis (Dr Fred Hrusa, pers. comm.; Ryan and Ayres 2000). One hundred seeds from one or more plants per site were placed on damp filter paper in petri dishes. The Petri dishes were sealed in air tight bags and left for 1 to 7 days at 25$^{o}$C. Once seeds had germinated and the roots (not the radicle) were 0.5 to 5 cm in length, 10-15 seedlings were removed, placed in water at 4$^{o}$C and stored in a fridge at 4$^{o}$C for 18-24 hrs. Seedlings were subsequently hydrolyzed with 1 M HCl at 60$^{o}$C for 8 min and then stained with Feulgen stain for 2 hrs. The first 1-3 mm tip of each root was macerated and stained with FLP orcein on a glass slide. Cover slips were placed on the slide, and each slide was heated gently over a flame and squashed. Chromosome counts were made from multiple cells from the roots of ten seedlings from each collection site. Only cells with fully contracted, clearly observable chromosomes were used (Shepherd and Yan 2003). Representative images of mitotic cells at metaphase were recorded with a Zeiss AxioCam (MRm) microscope.

**Results**

Somatic chromosome counts were recorded for populations collected from W.A. and from *S. tragus* and *S. australis* from the USA. A count of 2*n* = 18 chromosomes was recorded for each of the four putative *Salsola* taxa found in Western Australia, as well as the sample of *S. australis* from the USA, and 2*n* = 36 chromosomes was recorded for the *S. tragus* population from the USA (Table 4.2, Figure 4.1).

Table 4.2: The somatic chromosome number of the putative taxa of the *Salsola* genus from Western Australia, as well as *S. tragus* and *S. australis* from the USA. The table includes the site number, the number of seedlings from which chromosome assessments were made (N), the population type, (as identified by Chapter 3), collection location (including name of the region and GPS coordinate, WGS84) and chromosome number.

| Site | N | Type | Location | Latitude | Longitude | 2*n* |
|---|---|---|---|---|---|---|
| 2 | 11 | D | Chapman Valley | -28.5517 S | 114.5654 E | 18 |
| 4 | 10 | *S. australis* | Chapman Valley | -28.4599 S | 115.1069 E | 18 |
| 5 | 10 | D | Greenough | -28.8015 S | 114.6178 E | 18 |
| 6 | 10 | C | Mullewa | -28.6908 S | 115.1509 E | 18 |
| 6 | 12 | C | Mullewa | -28.6908 S | 115.1509 E | 18 |
| 7 | 10 | *S. australis* | Mullewa | -28.6275 S | 115.3560 E | 18 |
| 8 | 10 | *S. australis* | Morawa | -28.8351 S | 115.7828 E | 18 |
| 10 | 10 | B | Yalgoo | -28.2989 S | 117.1394 E | 18 |
| 11 | 15 | B | Mt Magnet | -28.1104 S | 117.7990 E | 18 |
| 12 | 13 | B | Mt Magnet | -27.7875 S | 117.9189 E | 18 |
| 12 | 12 | B | Mt Magnet | -27.7875 S | 117.9189 E | 18 |
| 13 | 10 | B | Yalgoo | -29.0519 S | 117.7463 E | 18 |
| 14 | 10 | B | Yalgoo | -29.5166 S | 117.1732 E | 18 |
| 17 | 10 | D | Dandaragan | -30.2861 S | 115.0458 E | 18 |
| 18 | 10 | C | Wagin | -33.3024 S | 117.3923 E | 18 |
| 18 | 12 | C | Wagin | -33.3024 S | 117.3923 E | 18 |
| 19 | 10 | *S. australis* | Lake Grace | -33.1285 S | 118.4831 E | 18 |
| 20 | 11 | *S. australis* | Lake Grace | -33.0954 S | 119.0676 E | 18 |
| 20 | 14 | *S. australis* | Lake Grace | -33.0954 S | 119.0676 E | 18 |
| 21 | 10 | D | Dandaragan | -30.5109 S | 115.0690 E | 18 |
| 22 | 10 | D | Dandaragan | -30.5641 S | 115.1066 E | 18 |
| 25 | 11 | *S. australis* | Merredin | -31.5026 S | 118.2277 E | 18 |
| 25 | 12 | *S. australis* | Merredin | -31.5026 S | 118.2277 E | 18 |
| 26 | 10 | *S. australis* | Kalgoorlie | -30.7342 S | 121.4670 E | 18 |
| USA | 10 | *S. australis* | Santa Nella, CA | -37.0619 N | 121.0167 W | 18 |
| USA | 10 | *S. tragus* | Davis, CA | -38.5472 N | 121.7122 W | 36 |

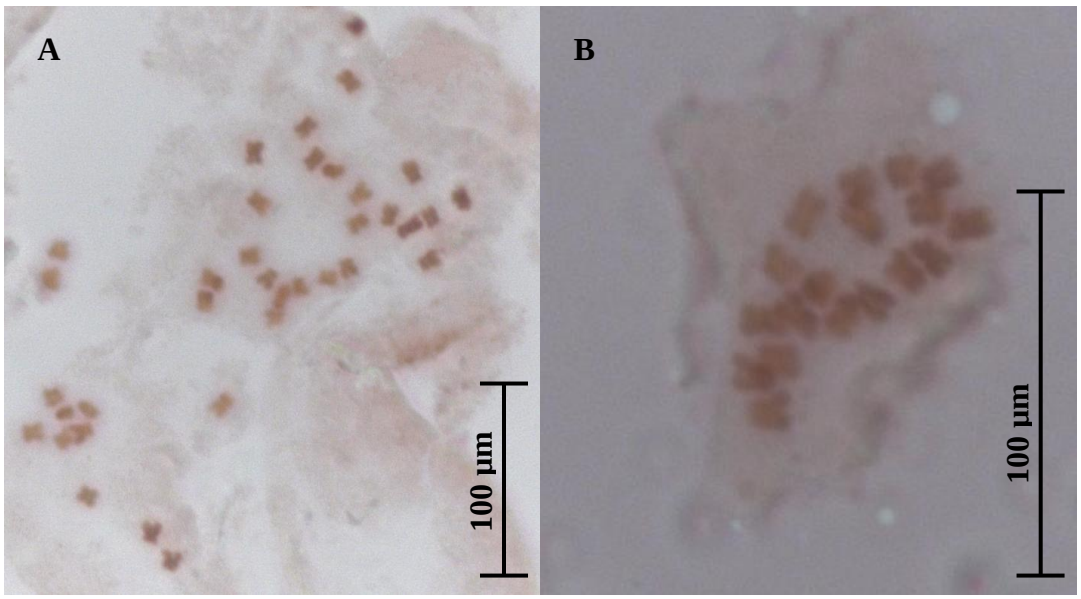


Figure 4.1: Somatic chromosomes from a root tip squash of (A) *Salsola tragus* from the USA: 2*n* = 36 chromosomes and (B) *Salsola australis* from plant 1, site 5: 2*n* = 18 chromosomes.

**Discussion**

All putative *Salsola* taxa within southwest Australia have 2*n* = 18 chromosomes, and the *S. tragus* from the USA had 2*n* = 36 chromosomes. This evidence indicates that Western Australian taxa are not *S. tragus*, but rather an alternative, diploid species of the genus *Salsola* (Table 4.1). This cytological and genetic evidence (Chapter 3), combined with the prior morphological comparison of Australian material with the *S. australis* lectotype, resulted in the reclassification of the common Western Australian wheat-belt weed variety of *S. tragus* subsp. *tragus* to *S. australis*. Voucher specimens of *S. tragus* subsp. *tragus* at the Western Australian herbarium that morphologically resemble *S. australis* have been reclassified and the species is recognised on the herbarium website (Western Australian Herbarium 2007). *Salsola australis* is mainly found in the wheat-belt (Geraldton to Esperance), but isolated populations are distributed throughout the central and northern regions of the state (Figure 4.2). The three remaining putative taxa of the genus *Salsola* are diploid, and genetically similar to *S. australis*. However, alternative classification of these species is still under consideration, and so these taxa are still labelled *S. tragus* subsp. *tragus* (Western Australian Herbarium 2007). *Salsola tragus* subsp. *grandiflora* is also a recognised species in Western Australia (Western Australian Herbarium 2007). This taxon from

northern Australia, which is endemic to Australia, was not available for inclusion in this research or the prior genetic research.

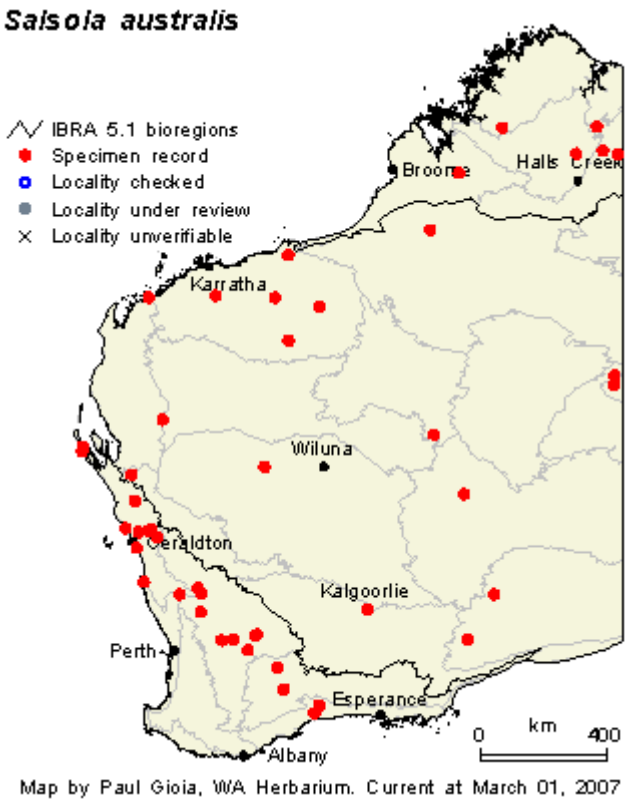


Figure 4.2: The distribution of *S. australis* in Western Australia (Western Australian Herbarium 2007). Mapping by Paul Gioia. Image used with the permission of the Western Australian Herbarium, Department of Environment and Conservation (http://florabase.calm.wa.gov.au/). Accessed on Friday, 23 March 2007.

Morphologically, *S. tragus* is distinguished from *S. australis* predominately by the fruiting perianth (Dr Fred Hrusa, pers. comm.). The morphological analysis of the genus *Salsola* conducted by Rilke (1999a) included an investigation of populations found throughout the entire continent of Australia. Rilke (1999a) stated that the fruiting structure of species of the genus *Salsola* found in Australia was similar to that of the original voucher specimen of *S. australis* produced by Robert Brown (1810). However, it was assumed that *S. tragus* and *S. australis* were the same species. Now that it is apparent that they are not the same species, the evidence presented by Rilke (1999a) implies that the species *S. australis*, and other taxa closely related to *S. australis*, are found throughout Australia. Further research of the genus *Salsola* in Australia is required to identify all taxa found here. Genetic analysis of these taxa, with comparison

to species of the genus *Salsola* from other countries (Rilke 1999a), may positively identify the origins of Australian species of the *Salsola* genus.

Reclassification of species of the *Salsola* genus in Western Australia and the determination of their origins has implications for the management of these plants in both natural and agricultural systems. Firstly, *S. tragus* is a highly invasive weed worldwide (Rilke 1999a) and since it is not currently found in Western Australia, ensuring this weed continues to be excluded should become a quarantine issue. Secondly, correct identification of the weedy agricultural species of the genus *Salsola* within Western Australia may improve management of these plants. While *S. australis* and *S. tragus* are morphologically similar, there are differences in the biology and ecology of these species that will affect management decisions (Mitchell and Wilcox 1988; Rilke 1999a; Young 1991). Thirdly, since the species *S. australis* was discovered in Australia first, at least 68 years before it was noted in the USA (Bentham 1870; Brown 1810; Young 1991), it is possible that these four putative taxa (including *S. australis*) are endemic to Australia rather than introduced. Currently these taxa are considered to be weeds of native vegetation and control is attempted in areas of protected vegetation. If they are native species they can be conserved in the Australian States in which they originated, although they will still need to be controlled where they have expanded into agricultural regions.

Chapter 3 and 4 have concluded that the predominant weed species of the *Salsola* genus in Australia is *S. australis*. As a result, all following work in this thesis is conducted on populations of *S. australis* (as opposed to the other variants of the genus *Salsola* found near and within the wheat-belt). The following three chapters discuss seed bank characteristics, seed dispersal and population ecology of *S. australis*.

# Chapter 5

# Characteristics of the seed bank of *Salsola australis* R.Br.[2]


**Abstract**

The seed biology, viability, germination temperature requirements and germination cues of the summer annual weed *Salsola australis* were determined for three ecotypes from the cropping region of Western Australia. Plants of the three ecotypes were collected from the Lake Grace, Merredin and Morawa regions. The three *S. australis* ecotypes were then grown in their original environment and in a common environment at the University of Western Australia. Average seed viability at plant maturity varied significantly between ecotypes, ranging from 7.6% and 9.8% for the Lake Grace and Merredin populations to 62.8% for the Morawa population. *Salsola australis* seed was able to germinate over a wide temperature range (5-37$^{o}$C), with 11-20$^{o}$C being the optimum range. Peak establishment periods varied between ecotypes with the Lake Grace ecotype establishing predominately in summer while the Morawa ecotype established primarily in winter. There was no peak period of establishment for the Merredin ecotype, which established throughout the year. Given that all ecotypes have the same temperature range for optimal germination, annual variation in times of peak establishment may be the result of ecotypic adjustments to the varying climatic conditions in the three districts. Each ecotype produced seeds with no dormancy and seeds that remained dormant for over a year. An average of 80.7% of seed from field plants and 32.3% of seed from UWA plants remained dormant in the year following seed production. Both the ecotype from which seeds are obtained and the precipitation levels in the maternal environment in which seeds develop influenced levels of seed viability and dormancy.


## Introduction

Summer growing annuals in southern Australian agricultural systems face a harsh and unpredictable environment. The Mediterranean type climate of this region consists of a cool, moist winter-spring growing season (May-September) interspersed with hot arid summer-autumn seasons. In this climate, rainfall is limiting and highly variable both

[2] Part of the information presented in this chapter was published as a conference paper, titled 'Seed viability and dormancy in roly poly (*Salsola tragus* L.) populations.'

between and within years and temperature and transpiration rates are high during summer and autumn. Research from both southwest Australia and other continents indicates that the most common strategy employed by annual plant populations to survive arid and unpredictable conditions is the establishment of a dormant soil seed bank (Baskin and Baskin 1989; Bell et al. 1993; Harper 1977). In particular, summer annuals commonly produce seeds with innate dormancy, which ensures that seeds do not germinate immediately following seed dispersal, but remain in the seed bank until there are favourable growing conditions (Baskin and Baskin 1989; Mott 1972; Silvertown 1987). This trait has been found in several species within the *Salsola* genus that have established in disturbed habitats in the USA (Evans et al. 1982; Young 1991) and Europe (Ignaciuk and Lee 1980). Annuals usually also produce a proportion of seed with long term dormancy mechanisms, allowing the establishment of a long term seed bank to ensure the species persists beyond unfavourable seasons (Harper 1977; Silvertown 1987). Seed bank life of summer annual species varies widely from less than one year to several decades (Table 5.1).

Table 5.1: Seed bank longevity of several summer annual weeds, including *Salsola* species, classified according to the scheme of Fenner and Thompson (2005). Seed banks are classed as transient (less than 1 year), short-term persistent (at least 1 year, less than 5 years) and long-term persistent (at least 5 years).

| Seed bank classification | Plant species | Location | Length of dormancy | Reference |
|---|---|---|---|---|
| Transient or short-term persistent | *Salsola kali* L. | USA, Europe | 2-3 yrs, <5 yrs in a 24$^{o}$C laboratory | Evans and Young (1972), Rilke and Reimann (1996) |
| | *Salsola rigida* Pall. | Iraq | <3 yrs | Al-Charchafchi et al. (1987) |
| | *Salsola vermiculata* L. | Syria, California | <1 yr or >3 yrs | Creager (1988), Sankary and Barbour (1972) |
| | *Salsola iberica* Sennen & Pau. | USA | 2-3 yrs | Fowler et al. (1988), Young et al. (1995) |
| | *Salsola paulsenii* Litv. | USA | <3 yrs | Chepil (1946), Evans and Young (1980) |
| | *Sonchus arvensis* L. | Canada | 1-5 years | Lemna and Messersmith (1990), Roberts and Neilson (1981) |
| Short-term persistent | *Cenchrus longispinus* (Hack.) Fern. | Australia | 3 years | Mullen et al. (2005), Twentyman (1974) |
| | *Tribulus terrestris* L. | USA | 4-5 years | Whitson et al. (1996) |
| Short or long-term persistent | *Lactuca serriola* L. | Europe | >4 years | Salisbury (1961) |
| Long-term persistent | *Polygonum aviculare* L. | Alaska, Australia | >20 years | Conn et al. (2006), Moore and Wheeler (2002) |

While seed banks are useful for annual plants, they result in increased seed mortality and delayed reproduction, reducing the potential rate of population growth (Fenner and Thompson 2005). However, this is only the case if it is assumed that all seedlings that establish have an equal chance of survival. Seedlings germinating from non-dormant seed may face an unsuitable environment and experience higher mortality than seedlings from dormant seeds that only germinate in favourable conditions. Species that do not produce a long term seed bank can maximise population growth rate (through reduced seed mortality), but only if they have mechanisms to ensure that seedlings survive in spite of environmental conditions. One of the mechanisms to improve seedling survival is broad scale seed dispersal, a trait which has been identified as an alternative strategy to seed dormancy in many annual and perennial species in Britain and the USA (Rees 1993). Widespread seed dispersal ensures that seeds germinate in spatially separated environments, whereas seed dormancy ensures that seeds germinate in temporally separated environments. Both strategies ensure that the seedlings face heterogeneous environments and the likelihood of all seedlings facing an unsuitable environment is reduced (Fenner and Thompson 2005; Rees 1993). A second mechanism that is common in arid habitats to ensure annual seed production is the production of mainly non-dormant seeds that germinate over a broad temperature range. Providing germination requirements are met, these non-dormant seeds establish following any precipitation event that produces favourable soil moisture levels. This allows the species to survive in habitats where precipitation is low and unpredictable (Baskin et al. 1993). For this strategy to be successful, the seedlings produced are required to survive over a very broad range of climatic conditions.

Some of the summer annuals listed in Table 5.1, including species of the *Salsola* genus, establish transient or very short-term seed banks. However, agricultural weed species of the *Salsola* genus appear to have mechanisms that allow plants to produce seed on an annual basis rather than establishing a dormant seed bank. Widespread seed dispersal is a common trait in agricultural weed species of the *Salsola* genus, including *S. australis* (Mallory-Smith et al. 1993; Stallings et al. 1995; Warren 2001) (Chapter 6). Likewise, seed that germinates over a very broad range of temperatures in response to precipitation is a common trait of weed species of the *Salsola* genus (Allen 1982a; Dwyer and Wolde-Yohannis 1972; Evans et al. 1982; Young and Evans 1972; 1979). While the temperature range for germination of *S. australis* is unknown, this species

does germinate throughout the year in southwest Australia and is found in a very broad range of climates and environments (Mitchell and Wilcox 1988; Wilson 1984). Not establishing a long term seed bank is unusual for a summer annual species, but *S. australis* appears to have adaptations such as seed dispersal and broad climatic tolerance that reduce the likelihood of failed reproduction in any given year. As a result, *S. australis* may survive with a transient or short-term persistent seed bank.

*Salsola australis* is a common weed throughout Australian agricultural regions (Mitchell and Wilcox 1988; Rilke and Reimann 1996) and knowledge of seed bank dynamics will assist in the development of weed management strategies. The purpose of this study was to characterise the seed bank life of *S. australis* populations found in cropping environments of southwest Australia. In particular, the aim was to examine the possibility that in this region *S. australis* has a transient (one year) seed bank. Specifically, seed from three geographically separated populations of *S. australis* was produced in controlled and field conditions, to generate genetic and environmental variation between seed sources. Seed viability, optimal temperature requirements for germination and annual periods of peak establishment were measured, in order to provide data on cumulative seedling establishment in the year following seed production.

**Materials and methods**

*Salsola australis* plants were selected from three field locations, Morawa (S 29$^{o}$50.065' E 115$^{o}$58.190'), Merredin (S 31$^{o}$30.094' E 118$^{o}$13.397') and Lake Grace (S 33$^{o}$7.426' E 118$^{o}$28.592') (i.e. three ecotypes) in southwest Australia. The three populations were labelled as ecotypes because the genetic analysis (Chapter 3) indicated that the populations are genetically distinct. These sites are located in the northern, central and southern regions of the Western Australian wheat-belt. Seed was harvested from plants at each field site, and then grown at both the field site from which it was harvested (field population) and at the University of Western Australia, Nedlands Campus (UWA population), Perth W.A. (S 31$^{o}$59.041' E 115$^{o}$49.086') (Figure 5.1). Seed was harvested from the field and UWA populations produced from each of the three ecotypes and used in the seed viability, germination temperature requirement and seed bank longevity experiments detailed below.


Figure 5.1: Sites in Western Australia at which plants used in these experiments were collected and grown.

*Seed source – UWA grown populations*

Seeds were collected from the three *S. australis* populations at Morawa and Merredin in March 2005, and Lake Grace in December 2004. Each population was taken from a field that was in wheat production the previous winter-spring growing season. No herbicides had been applied during the summer period (December to March) in which the plants grew. At each location, 10-15 mature plants were cut at the base and manually crushed in a plastic tub, which released seed. Seeds from each ecotype were planted in 10 pots (194 mm diameter, 205 mm tall) filled with potting mix (50% mulched pine bark, 25% sand and 25% peat moss) on 20 January 2005 and placed in an outdoor netted enclosure at UWA. Seedlings were thinned to two plants per pot following establishment, and were fertilised and watered as required to ensure healthy growth. Populations from the three ecotypes were spatially separated, surrounded by pollen proof netting to prevent cross-pollination between populations, and allowed to grow to senescence before harvest occurred. In this manner, seeds of the three ecotypes were matured under identical conditions.

The Morawa population was harvested on 11 May 2005, the Lake Grace population on 30 May 2005 and the Merredin population on 7 June 2005, by cutting senesced plants at

the base of the stem and placing the above ground plant material in paper bags. The plants were threshed using a Perspex grain thresher (drum thresher with rubber beaters)[3] to release seed. Threshing did not release all seed, with some remaining firmly attached to the parent plant following senescence. The threshed material from each population was sieved to separate the easily shed seeds from the rest of the plant material, including the retained seeds.

*Seed source – Field grown populations*

Seed was harvested from field populations growing at Morawa on 15 March 2005, at Merredin on 31 March 2005, and at Lake Grace on 26 March 2005, and processed as described for the UWA grown plants. In contrast to UWA grown plants, the field plants grew under rain-fed conditions without the addition of water or fertiliser beyond what was naturally available at each site.

*Seed viability*

For each ecotype, bulked seed samples from 10 plants in the field population and 10 plants in the UWA population were taken after harvest. Three samples of 100 easily shed or retained seeds were removed from the bulked seed sample from each population. The seeds in each sample of 100 that had germinated on the mature plant prior to harvest were scored as viable and removed. The remaining seeds in each sample were placed in 10 cm petri dishes on wet filter paper (Whatman N$^{o}$ 3 filter paper). The petri dishes were sealed in plastic bags to retain moisture, and incubated in a growth cabinet with a 12 hr temperature cycle of 25/15$^{o}$C, under constant fluorescent light (50 µmol/m$^{2}$s$^{1}$). Each day for a week, germinated seeds were scored as viable and removed. The fruiting perianth (Figure 5.2) was removed from the remaining un-germinated seeds and they were placed in 1% 2,3,5-triphenyltetrazolium chloride (Sigma-Aldrich Co.) for 48 hr at 30$^{o}$C (TZ test) (International Seed Testing Association 1999). Solid purple embryos were recorded as viable seed. Very light pink, white or brown embryos were scored as non-viable.

*Germination requirements*

Seed harvested from each field and UWA grown population were placed in petri dishes on wet filter paper, with 100 seeds per dish replicated 3 times. The petri dishes were

[3]Kingaroy Engineering Works Pty. Ltd., 2/6 Industrial Ave Kingaroy 4610.

sealed in plastic bags and incubated in growth cabinets at constant temperatures of 5, 11, 20, 30, 37 and 50$^{o}$C (under fluorescent light: 50 µmol/m$^{2}$s$^{1}$ PAR). Germinated seeds were counted and removed daily for 1 week. Total seed germination between populations varied from 10-90% of the sample, due to large variation in seed viability levels. As a result of this variation, seed germination was calculated as a percent of viable seed for each population in order to allow comparison of germination between populations. This was done by using the seed viability data collected above to estimate the number of viable seeds used in this experiment. The seeds used in both experiments were obtained from the same source and used at the same time, so seed viability levels between the seed samples should have been similar, although not identical.

*Seed bank longevity*

Samples of easily shed seed were taken from bulked seed harvested from each population grown in the field and at UWA. Likewise, samples of retained seed were taken from bulked plant material from each population. Each sample consisted of 1000 seeds, replicated 3 times. Foam boxes (482 mm long by 320 mm wide by 160 mm tall) were filled to a depth of 120 mm with potting mix (50% mulched pine bark, 25% sand and 25% peat moss). The seed samples were spread evenly over the surface and covered with potting mix to a depth of 1 cm. The boxes were arranged in a randomised block design in an outdoor netted enclosure at UWA, on 16 June 2005, and exposed to natural precipitation (no artificial watering). Each week for 12 months, seedlings were counted and removed. Total establishment per month was recorded. The seed viability data collected above was used to estimate the number of viable seeds per sample. Using this approximation of seed viability, seedling establishment over the 12 month period was converted to establishment as a percent of the viable seed, in order to compare establishment between the different ecotypes. Rainfall data over this period was obtained from the Floreat Park weather station.

*Data analysis*

The seed viability data were analysed using an ANOVA (GENSTAT Version 8.2). Seed viability was compared for easily shed and retained seed, within and between ecotypes grown in both locations. Means were compared using LSD ($P < 0.05$). The germination requirement data were subjected to a square root and arcsine transformation and were analysed using an ANOVA, to compare seed germination between ecotypes grown in

both locations over different temperatures. Transformed means were compared using LSD ($P < 0.05$). The data and LSD values are presented as untransformed means. The seed bank longevity data was also subjected to a square root and arcsine transformation. An ANOVA was used to compare establishment between ecotypes, location in which the population was grown, seed type (easily shed or retained seed) and time. The transformed means were compared using LSD ($P < 0.05$). The data is presented as untransformed means.

*Climate data for locations of seed production*

During the year in which the seeds were produced, the UWA site experienced higher precipitation (700 mm) compared to that of the field sites (228 mm at Morawa, 292 mm at Merredin and 374 mm at Lake Grace). The long term averages for the field sites indicate that in the year of seed production, Morawa and Merredin experienced below average rainfall, whereas rainfall was above average at Lake Grace (Table 5.2).

The long term climate data indicates that the district of Lake Grace has higher rainfall and lower average temperatures over summer than Merredin or Morawa. As a result, evaporative demand is lower in summer and periods of moisture stress experienced by summer growing annuals are shorter in this district. Merredin has higher summer temperatures than Lake Grace and low annual rainfall. Morawa has the highest average summer temperatures. High evaporative demand over summer at Merredin and Morawa, combined with lower rainfall, indicate that summer growing plants in these districts would be exposed to longer periods of moisture stress. Winter temperatures at Lake Grace and Merredin are cooler than those at Morawa.

Table 5.2: The average daily maximum and minimum temperature and average total monthly rainfall at Lake Grace, Merredin Research Station and Morawa, from 1914 to 2004. Climate data was obtained from the Bureau of Meteorology, Western Australia.

| Month | Lake Grace | | | Merredin | | | Morawa | | |
|---|---|---|---|---|---|---|---|---|---|
| | Max temp (ºC) | Min temp (ºC) | Rainfall (mm) | Max temp (ºC) | Min temp (ºC) | Rainfall (mm) | Max temp (ºC) | Min temp (ºC) | Rainfall (mm) |
| Jan | 31.5 | 14.8 | 16.3 | 33.7 | 17.1 | 14.3 | 36.7 | 19.0 | 14.6 |
| Feb | 30.6 | 15.1 | 19.0 | 33.0 | 17.0 | 15.9 | 36.1 | 19.5 | 17.7 |
| Mar | 28.0 | 14.1 | 22.8 | 29.9 | 15.4 | 20.8 | 32.9 | 17.4 | 22.8 |
| Apr | 23.9 | 11.5 | 22.9 | 24.9 | 11.9 | 22.1 | 28.2 | 13.8 | 22.0 |
| May | 19.6 | 8.6 | 44.3 | 20.1 | 8.2 | 38.3 | 22.9 | 9.8 | 46.2 |
| Jun | 16.5 | 6.7 | 52.1 | 16.7 | 6.4 | 49.2 | 19.2 | 7.4 | 59.5 |
| Jul | 15.3 | 5.7 | 48.8 | 15.7 | 5.0 | 48.0 | 18.1 | 6.2 | 54.7 |
| Aug | 16.4 | 5.6 | 41.2 | 17.0 | 4.9 | 37.9 | 19.4 | 6.4 | 39.4 |
| Sep | 19.1 | 6.7 | 30.7 | 20.5 | 5.9 | 23.0 | 23.0 | 7.7 | 21.9 |
| Oct | 22.7 | 8.6 | 22.5 | 24.3 | 8.6 | 16.2 | 26.6 | 10.3 | 15.3 |
| Nov | 26.4 | 11.3 | 18.3 | 28.4 | 12.3 | 14.4 | 30.8 | 13.7 | 10.9 |
| Dec | 29.6 | 13.4 | 14.3 | 31.9 | 15.2 | 14.1 | 34.5 | 16.6 | 8.8 |
| Total rainfall (mm) | | | 353.2 | | | 314.2 | | | 333.8 |

**Results**

*Seed viability*

The fruit of *S. australis* has a single seed contained within a fruiting perianth, composed of five papery wings. Each seed has a thin seed coat containing a coiled embryo. Germination consists of the embryo uncoiling to emerge from the seed (Figure 5.2). Although the fruiting perianth shown has wings, heterocarpy is evident and seed cases with no wings were also found in each population (the proportion of each seed type was not investigated).

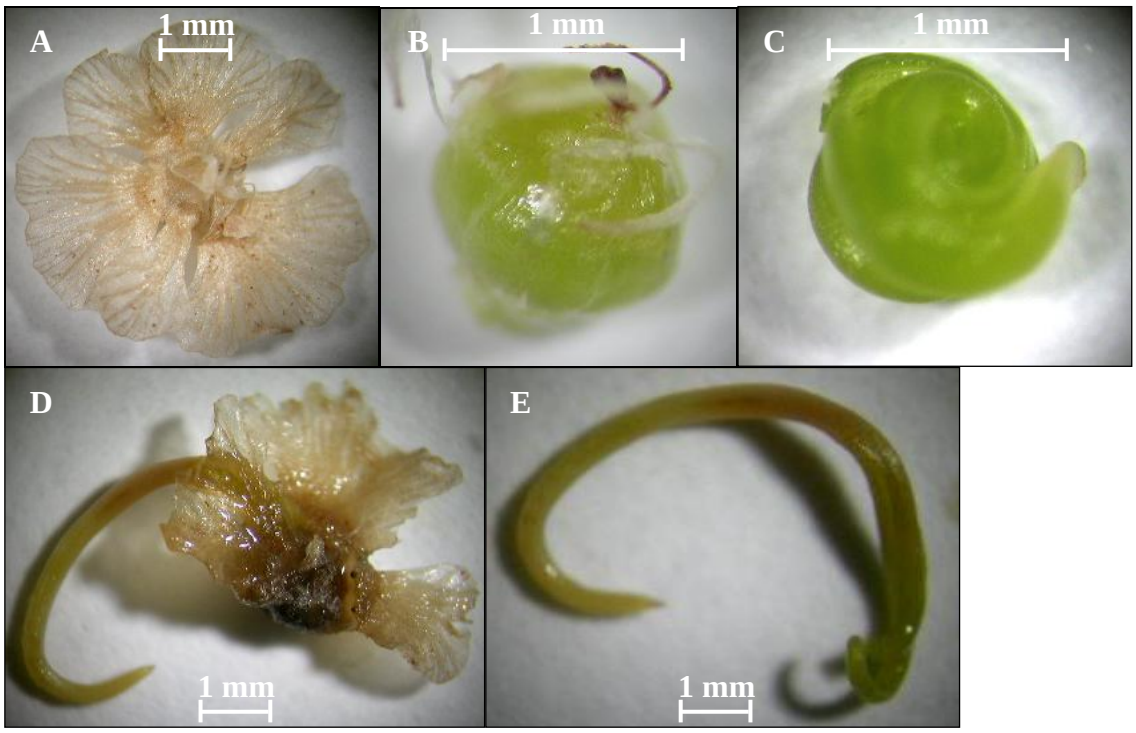


Figure 5.2: The fruiting perianth (A), seed (B), coiled embryo within the seed (C), embryo emerging from a seed still contained within the fruiting perianth (D) and fully emerged seedling (E) from a Lake Grace *S. australis* plant grown in the field.

When grown under irrigated conditions at UWA, germination of seed from fruit still attached to the plant was evident prior to and during plant senescence. This germination resulted from precipitation events that provided water directly to the seed, allowing them to imbibe while still on the plant. Irrigation events, which increased soil moisture availability but did not cause water to be directly applied to the seeds attached to the plants, did not trigger germination of attached seeds. Germination of attached seeds was not observed for field plants, presumably because no rainfall events occurred during the several weeks prior to harvest. For the Morawa UWA population, an average of 49% of easily shed seed and 15% of retained seeds germinated prior to harvest. For the Merredin and Lake Grace UWA populations, both the easily shed seed and the retained seed experienced similar germination. Total seed germination prior to harvest was 2% of seed from the Merredin UWA population and 0.5% of seed from the Lake Grace UWA population. For all populations, the youngest seed was least likely to shed or germinate before harvest, and usually remained firmly attached even after the plants were threshed. Overall, germination of seeds prior to harvest was higher for the Morawa population than for the Lake Grace or Merredin populations.

The viability of seed from senesced, harvested plants, varied considerably due to ecotype and environment, but was not affected by seed type (Figure 5.3). Average viability of seed from the Morawa plants (62.8%) was significantly higher ($P < 0.001$, LSD: 2.47) than that of the Lake Grace (7.6%) and Merredin (9.8%) ecotypes. Morawa and Merredin UWA plants produced seed with higher viability than the field populations ($P < 0.001$, LSD: 1.191). However, the reverse was evident in the Lake Grace populations, even though Lake Grace UWA plants had a greater supply of resources (water, nutrients) than the field plants. There was no obvious reason for the remarkably low seed viability observed in Merredin field plants and Lake Grace UWA plants, i.e. herbicide application during seed production or pathogens. The Merredin field site had below average precipitation in the year in which seed was produced, but this was also the case for the Morawa site. Seed viability was not significantly different (P: 0.151, LSD: 0.0449) for easily shed or retained seed in any population.

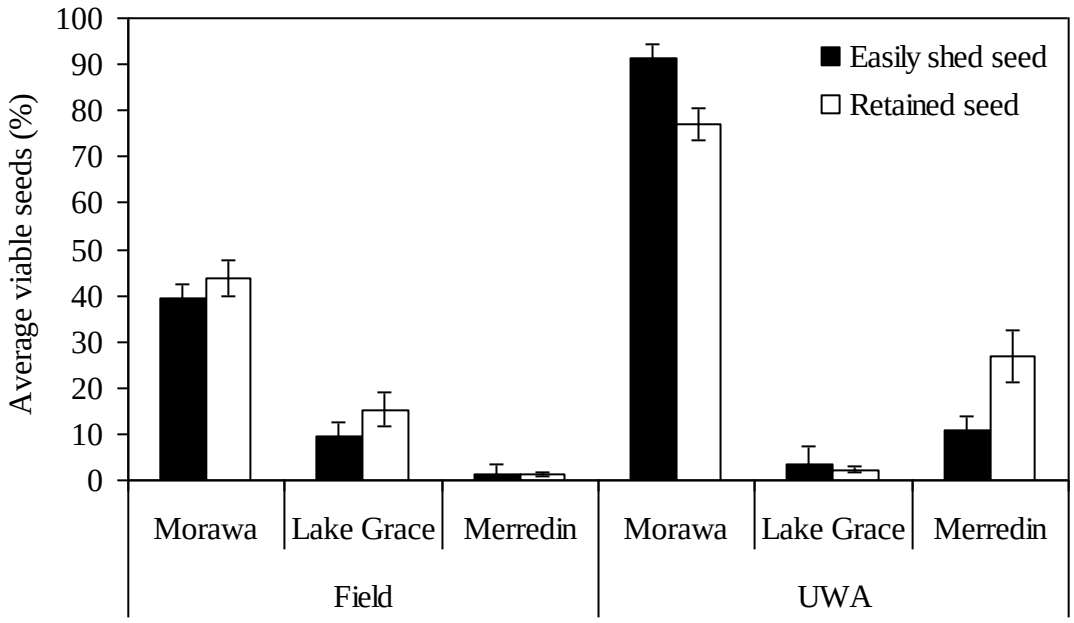


Figure 5.3: Average viability of easily shed and retained seed (in samples of 100 seeds) from the Lake Grace, Merredin or Morawa ecotypes, grown in the field or at UWA. Error bars represent the standard error of the mean from three replicates.

*Germination requirements*

Seeds from all three ecotypes germinated over a broad temperature range, from 5 to 37$^{o}$C (Figure 5.4). However, the optimal temperature for germination was 11-20$^{o}$C.

Germination was reduced at 5 and 30°C, and very low at 37°C ($P < 0.001$, LSD: 2.31). The Lake Grace field population was the only exception to this pattern, with germination at 5°C being equivalent to germination at 11°C. However, as for the other populations, germination of the Lake Grace seed was highest at 20°C (P: 0.002, LSD: 9.24). The upper temperature limit for germination was 37°C, as no germination occurred beyond this temperature for any population, but the lower limit for germination was not identified. The data from the Merredin field and Lake Grace UWA populations was not included in the analysis. Germination of these populations was very low due to low seed viability, and could not be analysed.

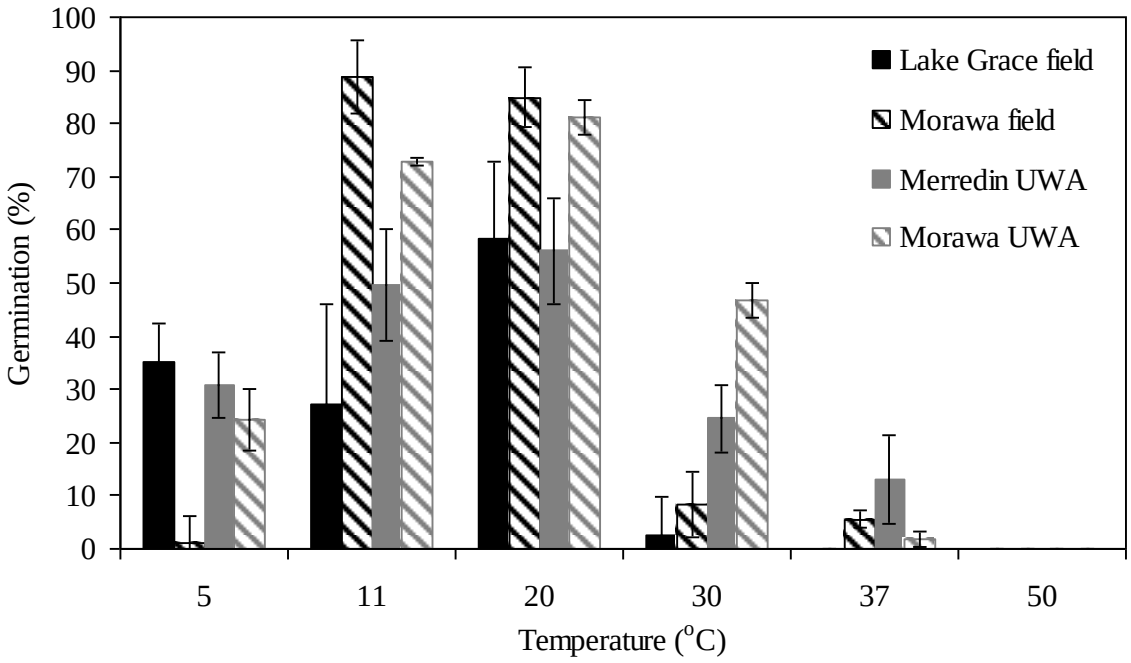


Figure 5.4: Seed germination (as a percent of the estimated viable seed in samples of 100 seed) from the Lake Grace field, Merredin UWA and Morawa field and UWA populations, over a range of constant temperatures. Error bars represent the standard error of the mean from three replicates.

*Seed bank longevity*

Over the 12 month monitoring period following seed production, cumulative establishment of each ecotype was influenced by environment and seed type (Figure 5.5). Establishment of seed produced by maternal plants grown at UWA was significantly higher ($P < 0.001$, LSD: 0.017) than that of seed from field grown maternal plants for all three ecotypes. The seed matured on UWA plants was supplied with

sufficient water and nutrients to ensure optimal growth. Further, natural precipitation at the UWA site was higher than either field site. Seed developed on field plants under the natural conditions of (limited) nutrient and water availability as extra inputs were not supplied. In both environments, cumulative establishment of easily shed seed was greater that that of retained seed, except that of the Merredin field population where the two were not significantly different ($P < 0.001$, LSD: 0.017). Total establishment as a percent of the viable seed were estimated values, based on the assumption that seed viability in these samples was equivalent to seed viability data obtained in the previous experiment. As a result, establishment of the Lake Grace UWA population (as a percent of viable seed) was 103%. Cumulative establishment over the year was equal between the three ecotypes (P: 0.081, LSD: 0.02).

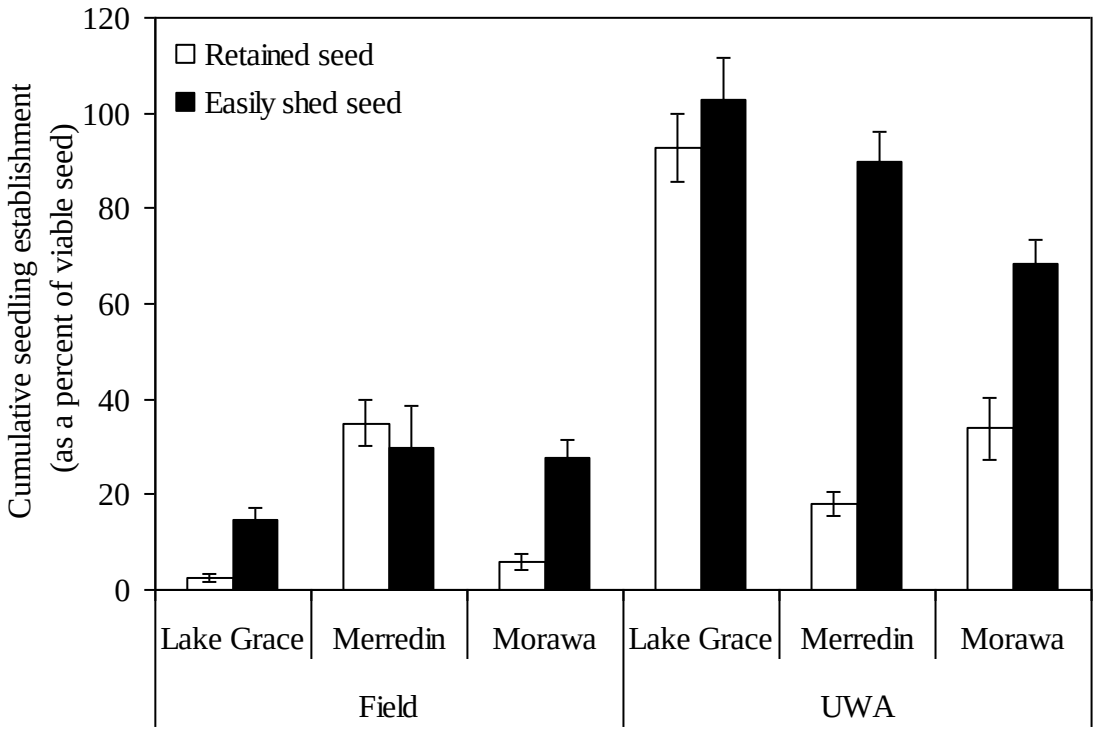


Figure 5.5: Cumulative seedling establishment from June 2005 to May 2006 of seed from the Lake Grace, Merredin or Morawa ecotypes, grown in the field or at UWA (as a percent of the estimated viable seed in samples of 1000 seeds). Error bars represent the standard error of the mean from three replicates.

During the 12 month monitoring period, times of peak seedling establishment occurred during July, January and April, but significant differences in peak establishment times

were evident between ecotypes (P < 0.001, LSD: 0.041). Seed from the Lake Grace UWA and field populations predominately established in the summer and autumn following seed production (January to April), although the field population also established in July and August (Figure 5.6). Morawa field and UWA grown seed experienced peak establishment immediately following seed production, in June and July, and then smaller establishment peaks in January and April for field grown seed (Figure 5.8). Seed from Merredin UWA populations established throughout the year, experiencing significant establishment from June to September, November, January and April. However, establishment was highest in July, January and April (P < 0.001, LSD: 0.071, Figure 5.7). The results from the Merredin field seed were not presented because establishment was too low for comparison between months.

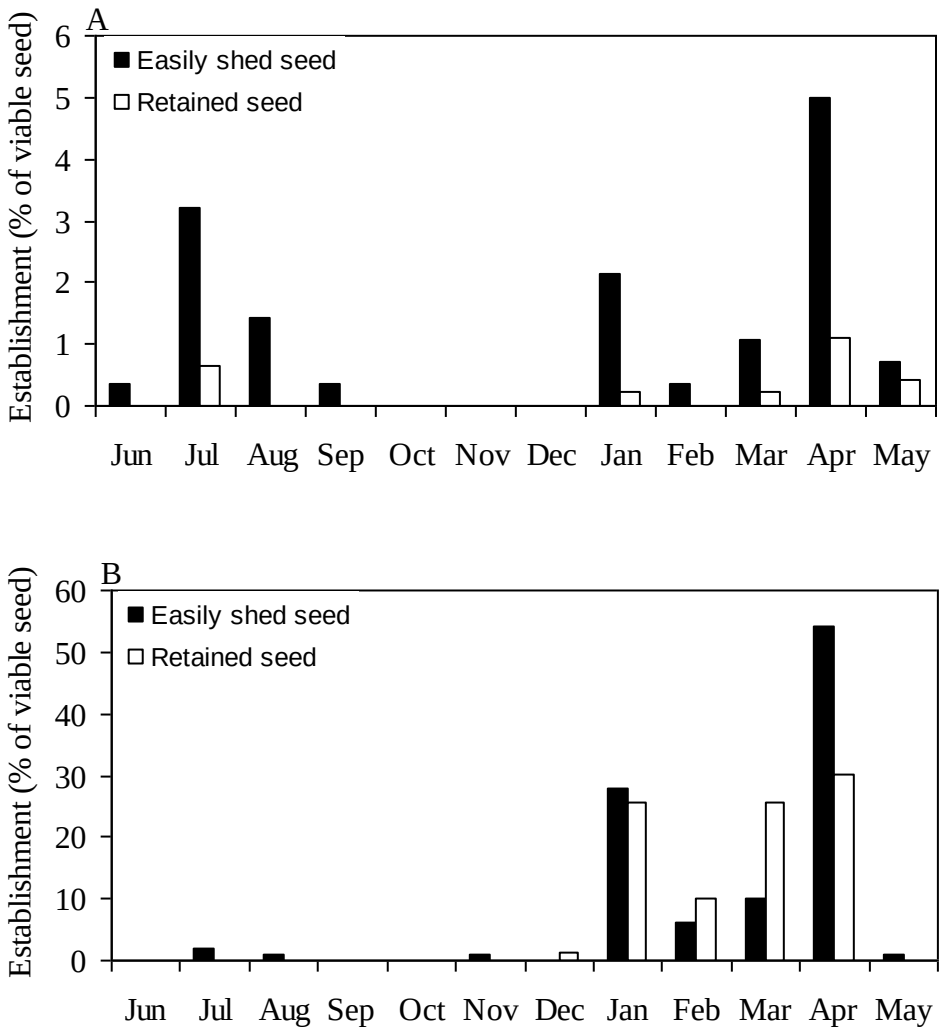


Figure 5.6: Total seedling establishment as a percent of the viable seed for the Lake Grace field (A) and UWA (B) population, from June 2005 to May 2006.

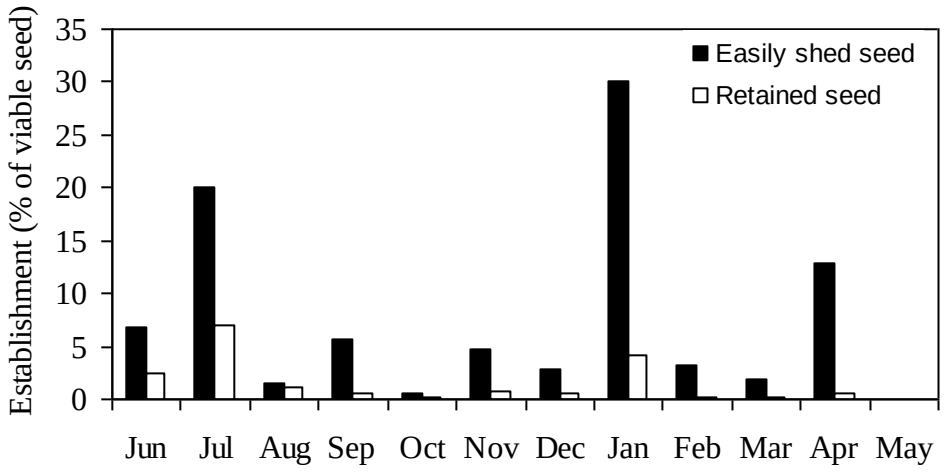


Figure 5.7: Total seedling establishment as a percent of the viable seed for the Merredin UWA population, from June 2005 to May 2006.

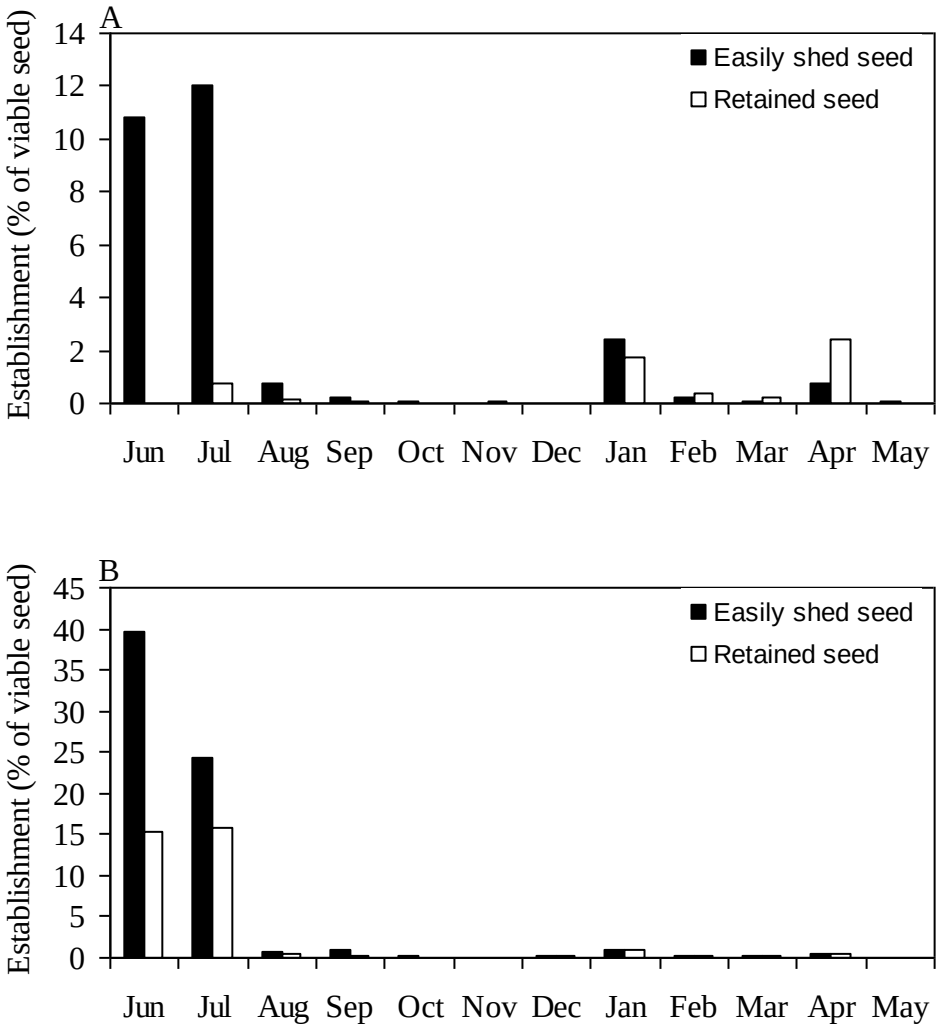


Figure 5.8: Total seedling establishment as a percent of the viable seed for the Morawa field (A) and UWA (B) population, from June 2005 to May 2006.

**Discussion**

*Salsola australis* appears to have a short-lived seed bank, but seeds did not all establish in the year following seed production. The proportion of seed remaining dormant was mainly influenced by the environmental conditions experienced by the maternal plant. Plants grown in controlled conditions at UWA, with a reliable and ample water and fertiliser supply, produced a high proportion of transient seeds. Plants grown in field conditions, where resources were limiting, produced a high proportion of seed that remained dormant for at least a year. The result of altered seed dormancy levels is that populations of *S. australis* in stressful, resource limited locations establish a dormant seed bank to ensure the population against years of low seed production. In locations where resources are not limited, dormancy is minimised to maximise the population growth rate (Fenner and Thompson 2005). Altered seed dormancy as a result of the seeds maternal environment has been observed in other annual weed species, such as *Lolium rigidum* (Steadman et al. 2004) and *Sinapis arvensis* (Luzuriaga et al. 2006). This trait has not been noted in other species of the *Salsola* genus, but altered seed dormancy as a result of the maternal environment would explain the variable and occasionally conflicting reports of seed dormancy in *Salsola* species (Creager 1988; Sankary and Barbour 1972; Young et al. 1995). The fate of seeds that remain dormant over the first year was not investigated. The dormant seed bank of other species of the *Salsola* genus lasts one to three years (Al-Charchafchi et al. 1987; Creager 1988; Evans and Young 1972; 1980). *Salsola australis* seeds greater than one year old may germinate at a later date, or lose viability.

All ecotypes produced retained seeds that were more likely to remain dormant than easily shed seeds in the year following seed production. Some level of dormancy is likely to be a pre-requisite for retained seed as they are not likely to successfully establish if they germinate whilst still attached to the plant. Burial is not essential for seed of *Salsola* species to successfully establish, but they do need to be pushed firmly against the soil surface or be lying on very recently disturbed soil for the spiralling action of the uncoiling (germinating) embryo to push the radical into the soil (Wallace et al. 1968; Young and Evans 1979). The retained seeds were predominately the youngest seeds on the plant and so their germination may be inhibited by immaturity. However, these seeds are found on the outer branches and branch tips, and are most likely to be released through abrasion with the soil surface during the tumbling motion

of the mobile plants (Mallory-Smith et al. 1993; Young 1991). Further, within the southwest Australian agricultural system, mature *S. australis* plants are often crushed and incorporated into the soil through cultivation at crop sowing (Mussell and Stewart 2004). As a result, much of the retained seed enters the soil seed bank. These seeds have equal viability and a higher likelihood of being dormant compared to the easily shed seeds, and likely influence both seedling recruitment and the size of the dormant seed bank. Physically removing or burning senesced plants (as opposed to crushing them through cultivation) to remove the viable, dormant seeds they retain would reduce the proportion of dormant seed entering the soil seed bank.

Seed from the three ecotypes experienced different periods of optimal establishment throughout the year when grown at UWA, which likely reflects genetic adaptations to the climate of the region in which each ecotype originated. The bulk of seeds from the Lake Grace ecotype established over summer and early autumn (January to April). Lake Grace experiences cooler summer and autumn temperatures and higher rainfall than the northern sites. This would result in lower evaporative demand and would ensure that *S. australis* plants germinating in summer would be subject to shorter periods of moisture stress than at the other locations. However, the average minimum winter temperatures are below the temperature range for optimal germination (i.e. 11-20$^{o}$C), and so germination in winter is probably inhibited. Seed from the Morawa ecotype established in winter, but winter is more suitable for seedling establishment than summer in this district. The average winter temperatures are close to the optimal temperature range for *S. australis* germination. The average temperature in summer is higher than at Lake Grace, and average summer rainfall is low, resulting in longer periods of moisture stress for summer annual plants. Seed from the Merredin ecotype established at low levels throughout the year, but at Merredin neither winter nor summer presents optimal conditions for germination. Winter minimum temperatures are below the optimal temperature for germination as at Lake Grace, and summer is hot and dry as at Morawa. Annual rainfall at Merredin is lower than at either of the other sites. The low rainfall may explain why seedlings from the Merredin population experienced significant seedling establishment throughout the year. Production of non-dormant seeds to take advantage of all precipitation events is a strategy recorded in other species found in arid environments with unpredictable rainfall, i.e. *Sonchus arvensis* (Baskin et al. 1993; Lemna and Messersmith 1990).

Establishment of multiple cohorts of *S. australis* throughout the year may have resulted from short-term dormancy, as well as adaptations to climatic conditions. In other species of the *Salsola* genus, short-term dormancy results from after-ripening (i.e. germination at a wider range of temperatures following exposure to cool winter temperatures) (Allen 1982a; Dwyer and Wolde-Yohannis 1972; Evans et al. 1982; Young and Evans 1972; 1979). After-ripening was not investigated here, as it was initially determined that some populations (Morawa) had no short term dormancy, and the optimal temperature for germination of all populations could occur throughout the year in Australia. Delayed germination of the Lake Grace seed and a portion of the Merredin seed may have resulted from after-ripening requirements as had been recorded for other species of the *Salsola* genus, but was clearly variable between ecotypes and environments.

Seed viability mainly varied as a result of ecotypic (genetic) rather than environmental variation. The viability of the Lake Grace and Merredin seed was significantly lower than that found in seed from the Morawa populations, in both the field and UWA environment. Further, environmental factors that commonly result in low viability, such as low resource availability, pathogens or herbicide application, did not affect populations grown at UWA. The wide range in seed viability of *S. australis* ecotypes confirms the findings of Chapter 3. Viability of the 26 populations collected for genetic analysis ranged from less than 1% to 85%. Populations may be expanding in spite of low viability, through high fecundity and efficient seedling recruitment. However, low seed viability is not a common feature of the *Salsola* genus. Seed viability in morphologically similar species such as *S. kali*, *S. paulsenii*, *S. iberica* and *S. vermiculata* ranged from 90 to 100% (Creager 1988; Dwyer and Wolde-Yohannis 1972; Fowler et al. 1988; Young and Evans 1979), although viability of *S. pestifer* seed in Canada was only 44% (Crompton and Bassett 1985).

The low seed viability of the Merredin and Lake Grace populations probably results from a genetic abnormality. Genetic analysis indicated that these ecotypes were genetically dissimilar to the other populations of *S. australis* within the Western Australian wheat-belt, although ploidy levels were uniform (Chapter 3 and 4). Viability is so low that it would be reasonable to assume that these populations are approaching extinction. However, while it is assumed that low viability results in extinction, there

are multiple examples of random mutations that increase fitness in spite of low viability (Arnold et al. 1999; Emms and Arnold 1997; Wang et al. 1997). This is especially evident in species that require genetic diversity to adapt to changing environmental conditions (Arnold et al. 1999; Grant and Grant 1993). These populations of *S. australis* are agricultural weeds, and so their environmental conditions would change constantly due to implementation of weed control practices. Since these plants are successful (i.e. economically detrimental) weeds (CPD Borger, personal observation), the selection pressure that has allowed these plants to adapt to the agricultural practices of the region has maintained fitness in spite of low seed viability.

Weed management practices used against *S. australis* will need to vary between ecotypes due to the variation in annual periods of peak establishment. Seed from Lake Grace plants predominately established over several months in summer. These summer growing plants likely produce fewer seeds due to low availability of resources in summer (i.e. precipitation, soil fertility). However, the low, unpredictable summer rainfall results in the establishment of many seedling cohorts over summer and autumn. It would be difficult to control all seedlings with herbicides unless residual herbicides were used. Seeds from Morawa plants established in winter. Winter rainfall is both higher and less erratic than summer rainfall, which may explain why seedlings all established within a two month period. Plants establishing in winter may produce more seed due to the higher resource availability in winter. However, emergence is correlated with the winter cropping period, and *S. australis* could be controlled by in-crop herbicides. Seed from Merredin plants established throughout the year. The establishment of seedlings at variable times of the year makes it difficult to apply herbicides to each cohort. However, the lack of sustained periods of optimal germination temperatures and lower precipitation indicate that Merredin is a less suitable environment for this weed. This may explain why *S. australis* is a minor weed in this district and not the more severe problem that it is in Morawa or Lake Grace. The ecotypic variation evident between populations of *S. australis* is likely to be part of the reason that this species occupies such a diverse range of environments in Australia (Mitchell and Wilcox 1988; Rilke 1999a; Wilson 1984).

# Chapter 6

# The mobile seed bank of *Salsola australis* R.Br.[4]

**Abstract**

*Salsola australis*, like other agricultural weed species of the *Salsola* genus, produces a mobile seed bank. Aspects of this mobile seed bank, including total seed production, rate of seed shedding, rate at which seeds lose germinability and the distance and directionality of plant movement, were investigated in three field trials. Total seed production was highly variable (ranging from 138 to 7734 seeds per plant) but was directly related to aboveground plant biomass at maturity. Following senescence, mature plants broke free of their root system and the wind driven plants moved considerable distances (1.6 – 1247.2 m). Half of the mobile plants moved less than 100 m as they became entangled with other *S. australis* plants within the stand. Seed shedding commenced before the plants became mobile and increased with movement, but was also related to the aging and weathering processes experienced by stationary or mobile plants. All plants retained a proportion of their seed in spite of movement, weathering and aging, although germinability of retained seed dropped to less than 2%, two months after maturity (i.e. a decline of 79%). *Salsola australis* engages in broad scale seed dispersal similar to that observed in other species of the *Salsola* genus, allowing this species to maintain a high rate of invasion and range expansion.

## Introduction

Broad scale seed dispersal is vital to the survival of early successional species, to ensure that seed reaches newly disturbed habitats in spatially separated areas (Wali 1999). Broad scale cropping agricultural habitats, with their annual disturbance regimes, allow these early successional species to form long term populations that do not rely as heavily on seed dispersal for survival. However, these species retain the ability for long distance seed dispersal in agricultural habitats, and so maintain a high rate of invasion and range expansion (Nathan and Muller-Landau 2000). Management of the seed bank and reduction of invasion rate are essential for the control of many annual weed species

[4] The information in this chapter has been accepted as a paper by the journal Weed Research, titled 'Tumbleweeds in the Western Australian cropping system: seed dispersal characteristics of *Salsola australis* R.Br.'

in agricultural habitats, but control becomes more complex for weed species with efficient, broad scale seed dispersal mechanisms.

Species of the *Salsola* genus that are agricultural weeds are often early successional species that engage in broad scale seed dispersal (Mallory-Smith et al. 1993; Schmidt and Reeves 1989; Stallings et al. 1995). Serotiny, a phenomenon where viable, dormant seed is retained on the plant for an extended period, rather than being immediately released to enter the soil seed bank is characteristic of these species (Fenner and Thompson 2005). When *Salsola* plants senesce, a specialised layer of cells at the base of the plant stem degrade and allow the above ground section of the plant to break free of the roots, enabling the plant (and retained seed) to become mobile (Young 1991). Pushed by the wind, tumbling plants may travel for 3-4 kilometres, releasing seed as they impact on the soil or other surfaces during the tumbling motion. The immigration of seed, facilitated by the mobile plants, may have a significant impact on seedling recruitment. The characteristics of the mobile seed bank that will affect the population ecology of this species include the extent and directionality of plant movement at the time of seed shedding, the rate of seed shed and the rate at which the seeds lose germinability.

Both the distance travelled and the direction of travel may depend on several environmental factors. Movement of *S. iberica* in Washington State (Mallory-Smith et al. 1993; Stallings et al. 1995) and *S. kali* in Idaho (Warren 2001) was influenced by climate (mainly wind speed and direction), vegetation type, geography and artificial obstructions. Plants travelled an average of 0.3-4 km, passing over fence-lines, field equipment, roadside ditches and wheat stubble, before they lodged in one of these obstacles securely enough to remain fixed for the remainder of the trial period.

The relationship between seed shedding and plant movement varies between species of the *Salsola* genus. *Salsola paulsenii* plants shed all seed before the plants become mobile, so distance moved by the plant has no relevance for seed dispersal (Young and Evans 1979). *Salsola pestifer* plants continuously shed seed after senescence, regardless of whether the plants are mobile or stationary (Crompton and Bassett 1985). *Salsola kali* plants shed very few seeds without vigorous movement of the plant (Young 1991). For those plants that retain seed during plant movement, rate of shedding depends on the

distance the plants travel and may depend on the age of the plants. Shedding increases with distance travelled, as the tumbling motion of the mobile plants shakes the seeds loose. Abrasion with the soil surface causes shedding of the seeds on the outer branches and causes the plant to break apart (Mallory-Smith et al. 1993; Young 1991). Increasing age of both the seeds and the plant may also cause shedding over time, whether movement occurs or not, as is the case for *S. pestifer*. Variation in shedding rate influences the proportion of long distance seed dispersal events that occur, as rapid shedding will ensure that seed do not disperse far from the point of origin. Possibly the survival of some species of the *Salsola* genus relies on rapid invasion of new, distant habitats to a greater extent than others, resulting in variation in seed dispersal strategies between species.

The rate at which seed in the mobile seed bank loses germinability will have a significant effect on the degree to which this seed reserve can influence population ecology, regardless of whether the plants are mobile or stationary. Young (1991) noted that seeds still attached on one year old *S. kali* plants were no longer viable. Germinability of seeds that remain attached to adult plants has not been determined for other species of the *Salsola* genus. However, within the soil seed bank, seeds of *Salsola* species generally remain viable for one to three years. For example, *S. kali* seed remains viable for two to three years with 60-90% germination in the first year (Evans and Young 1972), *S. paulsenii* seed remains viable for less than three years with 65% germination in first year (Al-Charchafchi et al. 1987; Evans and Young 1980) and *S. vermiculata* (Del.) Moq. seed germinates or loses viability in the first year (Sankary and Barbour 1972). The annual rate of seed germinability loss has not been investigated for many species of the *Salsola* genus, but a more in depth understanding of this factor will have an important effect on the most appropriate approaches for managing the seed bank.

*Salsola australis* (Chenopodiaceae), commonly known as roly poly or prickly saltwort, has been a weed of ruderal habitats throughout Australia for over 200 years and is economically important in the wheat-belt of Western Australia (Mussell and Stewart 2004; Rilke 1999a). In cropping systems it is a weed of the summer fallow period and depletes soil moisture and nutrient reserves, reducing the yield potential of subsequent winter crops (Mussell and Stewart 2004; Osten et al. 2006). Clearing mature plants

delays crop sowing, which also reduces yield potential (Tennant 2000). Within pasture systems, the prickly, mature plants damage stock and the young plants may cause oxalate poisoning (Jacob et al. 1992; Mussell and Stewart 2004). Senesced, mobile plants are a severe fire hazard and general nuisance (Mussell and Stewart 2004). Herbicide control is used in the cropping system, with limited success, but no management options are available in pasture systems (Mussell and Stewart 2004). To successfully manage this weed it is necessary to determine the impact mobile *S. australis* plants have on seed bank dynamics, and thus the invasion rate of this species. The characteristics of seed dispersal in *S. australis* have not previously been investigated. The impact of mobile plants on population ecology and invasion rate will be determined by the distance travelled by mature plants, rate at which shedding occurs and the rate of loss of seed germinability. The specific hypothesis addressed by this research is that *S. australis* will engage in broad scale seed dispersal, through gradual release of seeds retained on mobile plants.

**Materials and methods**

*Seed production*

Senesced *S. australis* plants that were not yet mobile were identified during the summer fallow period, in cropping fields at Lake Grace (S 33$^{o}$07.384' E 118$^{o}$28.568') on 12 February 2005 and at Morawa (S 29$^{o}$10.450' E 115$^{o}$52.220') on 7 April 2006. Lake Grace and Morawa are approximately 275 km southeast and 301 km north of Perth, Western Australia. At both sites the climate is mediterranean, although Lake Grace has cooler annual temperatures and higher average annual rainfall than Morawa (i.e. 353.2 and 333.8 mm). Soils at both sites consisted of yellow or white sands, near salt lakes or salt affected land. Twenty two mature plants from Lake Grace and 10 plants from Morawa were randomly selected and their height and width measured. The plants were excised at the base of the stem, individually crushed in a plastic tub and placed in paper bags. They were stored for one week in a 40$^{o}$C oven before weighing to determine above ground dry biomass. Plants were individually threshed and the number of seeds in ten 5 g sub-samples of threshed plant material was determined. These data were used to determine average seed number per gram of dry plant biomass for each plant. Total seed production was calculated using average seed number per gram of plant biomass and total plant biomass.

A linear regression analysis (GENSTAT Version 8.2) was used to correlate the parameters of plant size (height, width, volume or biomass) to seed production. The regression model was used in subsequent trials to estimate total seed production.

*Artificial wind experiment*

Thirty senesced plants were randomly selected in Morawa (S 29$^{o}$10.450' E 115$^{o}$52.220') on 7 April 2006. This date was selected because the weather was calm, with no wind. Plants were cut free at the base, weighed and randomly allocated into six treatment groups. The six treatments were 0, 50, 100, 500, 1000 and 5000 m distances of plant movement. For each treatment distance, the five plants were moved by an artificial wind (wind speed of 40 km/hr) generated with a leaf blower (Ryobi, 50-60 MHz). For the 500, 1000 and 5000 m treatments, the plants were pushed north for 100 m and then south for 100 m, which was repeated until the plants had moved the entire distance required by the treatment. To ensure that all plants were exposed to wind for a time span similar to that experienced by the plants that travelled 5000 m, plants in distances less than 5000 m were held stationary in plastic tubs (in order to catch any shed seed) and subjected to further wind from the leaf blower. In this manner, plants in 0, 50, 100, 500 and 1000 m treatment distances were exposed to wind for 16.7, 16.5, 16.3, 15 or 13.4 min. Each plant was weighed a second time to determine biomass loss. The plants were processed to determine the number of seeds remaining on each plant, using methods described above. Estimates of seed present on plants prior to movement were made using the regression model developed previously (using seed production data from both the Morawa and Lake Grace region). Seed remaining on the plant was compared to estimated total seed production to determine seed loss.

The experimental design was a replicated regression (Cottingham et al. 2005). Regression analysis was not appropriate so the data were subjected to an ANOVA (GENSTAT Version 8.2) to determine the effect of distance moved on seed and biomass loss. Means of seed and biomass loss were separated using least significant differences ($P < 0.05$).

*Seed shed over time*

Twenty-five plants that had emerged in November 2004 were selected at plant senescence in March 2005, from a population at Lake Grace (S 33$^{o}$14.507' E

118$^{o}$26.960'). Five plants were weighed and harvested at this time. The remaining plants were cut free, weighed and then tethered to a fence. They were removed over the following eight months by randomly selecting five plants every two months from May to November 2005. Following collection, plants were processed and the number of seeds per plant was estimated using procedures described above. Plant biomass at harvest was used to estimate total seed production at plant maturity. Seed remaining on the plant was compared to estimated total seed production to determine seed loss. Seed germinability was assessed by placing samples of 200 seeds from each plant in petri dishes on damp filter paper, incubating them at 25/15$^{o}$C under constant fluorescent light (50 µmol/m$^{2}$s$^{1}$ PAR) and measuring germination daily for two weeks.

The seed germinability data were subjected to a square root transformation to improve the distribution of the error variance, followed by an arcsine transformation. An ANOVA (GENSTAT Version 8.2) was used to compare remaining seed and seed germinability with plant age. Means were separated using least significant differences ($P < 0.05$).

*Natural movement experiment*

Sixty two mature plants were selected in a reasonably flat, uniform field at Morawa on 7 April 2006. The field contained no other vegetation besides wheat stubble and senesced *S. australis* plants. The selected plants were marked with spray paint and labelled, then excised at the base of the stem and weighed. Five plants were tethered to a stake at S 29$^{o}$10.450' E 115$^{o}$52.220' to prevent movement. The remaining 57 plants were placed un-tethered at this location and were allowed to move with the natural wind events. The point of release was in the centre of the area from which the 62 plants were selected, to ensure that the density of the *S. australis* population was not altered. An anemometer attached to a data logger (03001 Anemometer and Vane and CR10X Measurement and Control Module, Campbell Scientific Inc.) was established at the plant release point, with the anemometer 1 m above the ground. Maximum wind speed and average wind direction were recorded hourly for the duration of the trial. On 3 May 2006 (27 days after plant release) the plants were relocated, although 6 plants remained missing. The position of each plant was recorded using GPS and their distance from the starting point was determined. Each plant was collected and the seed number per plant was assessed

as above. The total initial seed number was estimated from initial biomass using the regression equation above and seed loss was determined.

A CDESCRIBE analysis (Fisher 1993; GENSTAT Version 8.2) was used to investigate direction of plant movement and wind events. The tests performed in a CDESCRIBE analysis are as follows: mean direction of travel, probability test of randomness and Rayleigh test (to indicate if the data were distributed uniformly and plants were equally likely to travel in any direction), Chi-square von Mises analysis (to indicate if plants predominately travelled in a single direction) and a kernel density estimate (to indicate if the data were uniform or if plants predominately travelled in one or more directions). Regression analysis was used to investigate the relationship between distance moved and seed or biomass loss.

**Results**

*Seed production*

Seed production of individual plants was significantly correlated to the above ground plant dry biomass (Figure 6.1). There was no difference in this relationship for plants at the two sites. The correlation was not significantly improved by incorporating plant height and width into the regression model ($r^2$ = 94.5%, $P < 0.001$), or by comparing seed production to plant volume ($r^2$ = 91.6, $P < 0.001$). The smallest plant collected had 3 g of dry biomass and produced 138 seeds. The largest plant was 110 g and produced 7734 seeds.

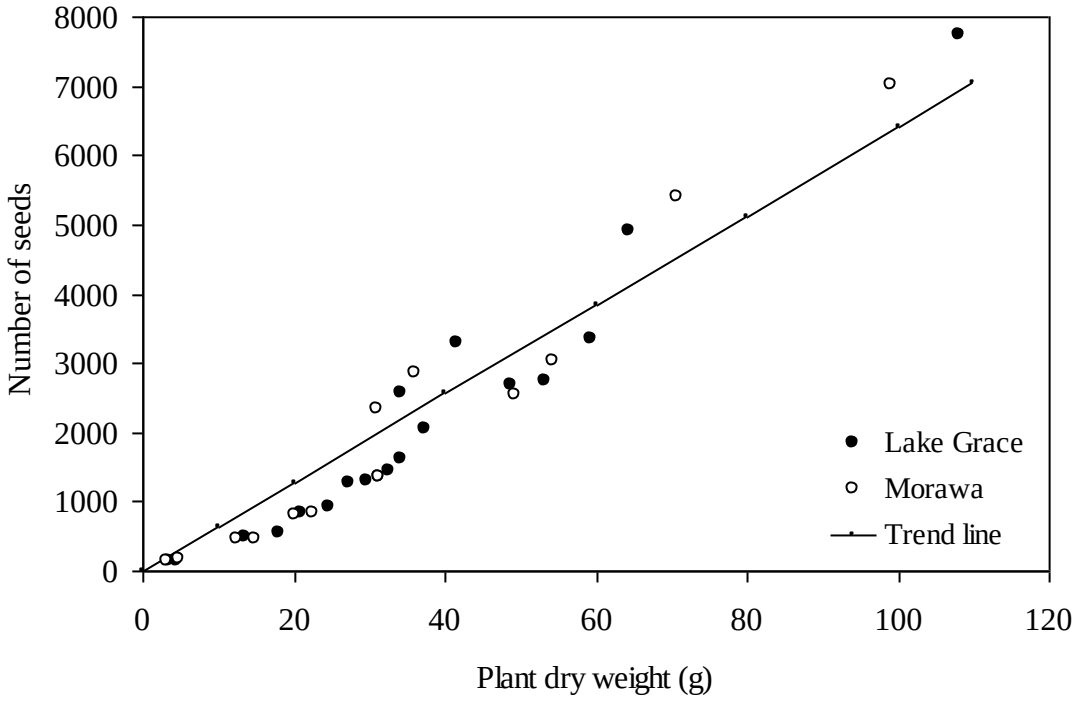


Figure 6.1: Total seed production and plant dry weight, for plants collected from the regions of Morawa and Lake Grace. The trend line indicates the relationship y = 64.01x ($r^2$ = 94.9%, P < 0.001).

*Artificial wind experiment*

Seed shedding began while the plants were stationary. Once the plants became mobile, shedding continued, but the amount of seed lost was not significantly greater than that of stationary plants until the plants travelled more than 500 m (Figure 6.2). The average seed lost from stationary plants was 24%, and plants that moved 500 m or less did not shed more seeds than plants that were stationary. Movement from 1000 to 5000 m increased average seed loss to 73%, significantly higher than the average seed loss of 32% for plants moving 0 to 500 m. Seed loss was mainly due to physical plant movement, not exposure to wind, since plants exposed to an artificial wind while held stationary in plastic tubs shed very little seed (CPD Borger, personal observation). Plant biomass was not reduced with increasing plant movement, since plants did not break apart, or even lose branches while travelling.

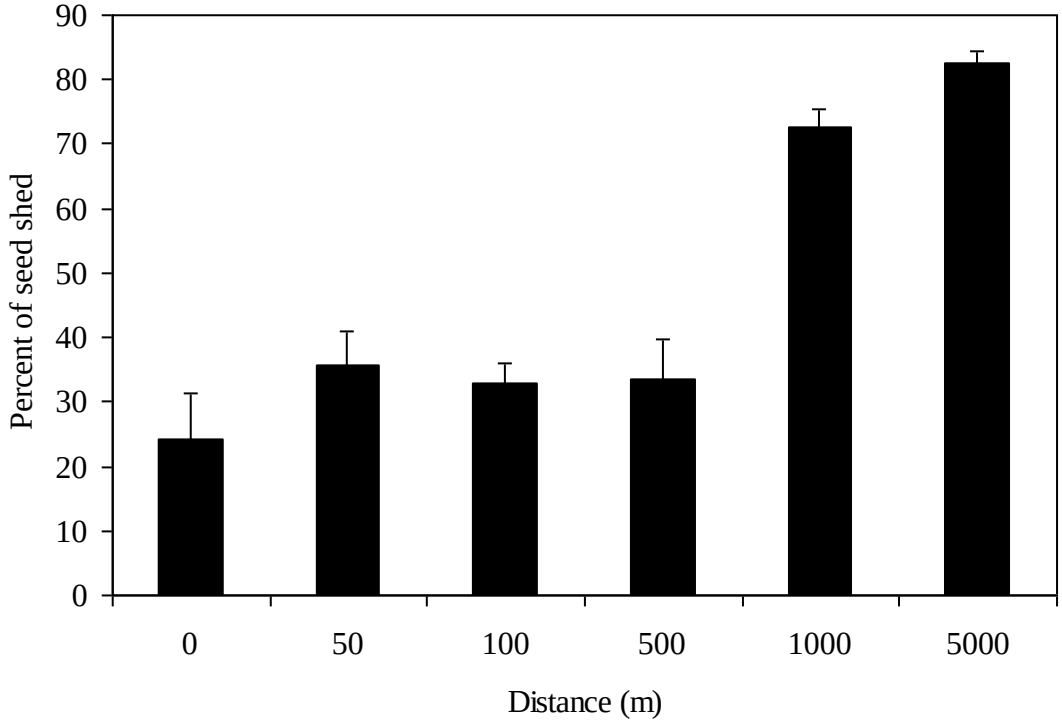


Figure 6.2: Seed shed, as a percent of the original seed number, and distance moved (0 to 5000 m) by each plant ($P < 0.001$, LSD: 10.25). Error bars represent the standard error of the mean from five replicates.

*Seed shed over time*

Few seeds were shed from plants that remained stationary throughout the year. The average number of seeds retained on plants harvested from March (time zero) to September gradually declined from 51 to 34 per gram of plant material (Figure 6.3). These values were not significantly different, and are within the range of seeds normally found on plants that have reached senescence, and are stationary. Thus for the first 6 months after maturation, seed shed very gradually from stationary plants. By November the plants had an average of 15 seeds per gram of plant material, which was significantly lower than for plants harvested in March and May, but not lower than for plants harvested in July or September. The average germination in March of 6.6% was significantly higher than later in the year where average germination had dropped below 2% (Figure 6.4).

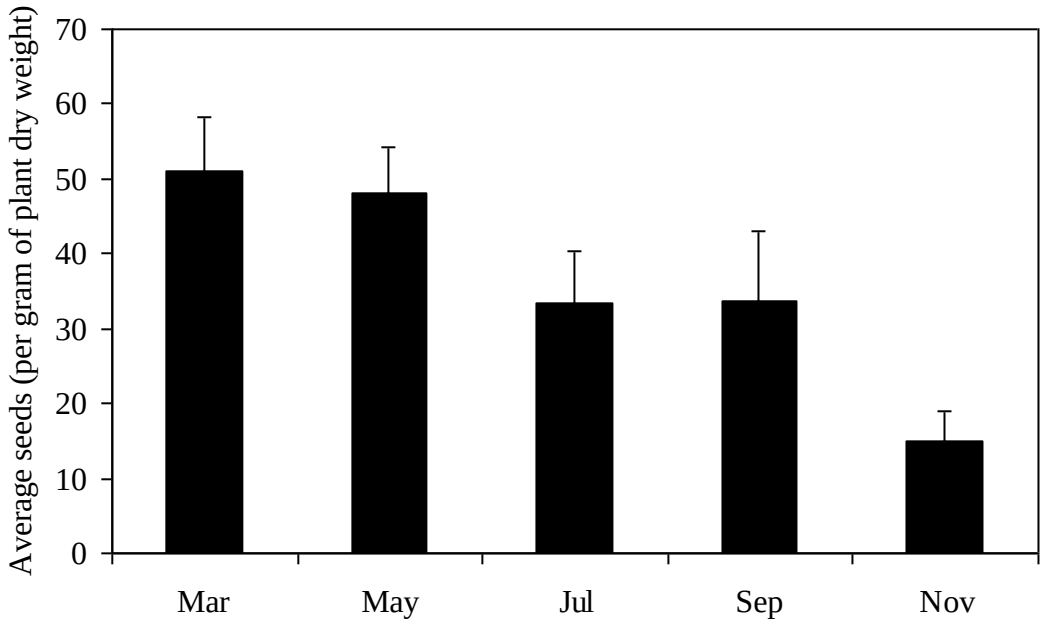


Figure 6.3: Average seeds per gram of dry plant biomass remaining on plants harvested from March to November, 2005 (P: 0.027, LSD: 22.66). Error bars represent the standard error of the mean from five replicates.

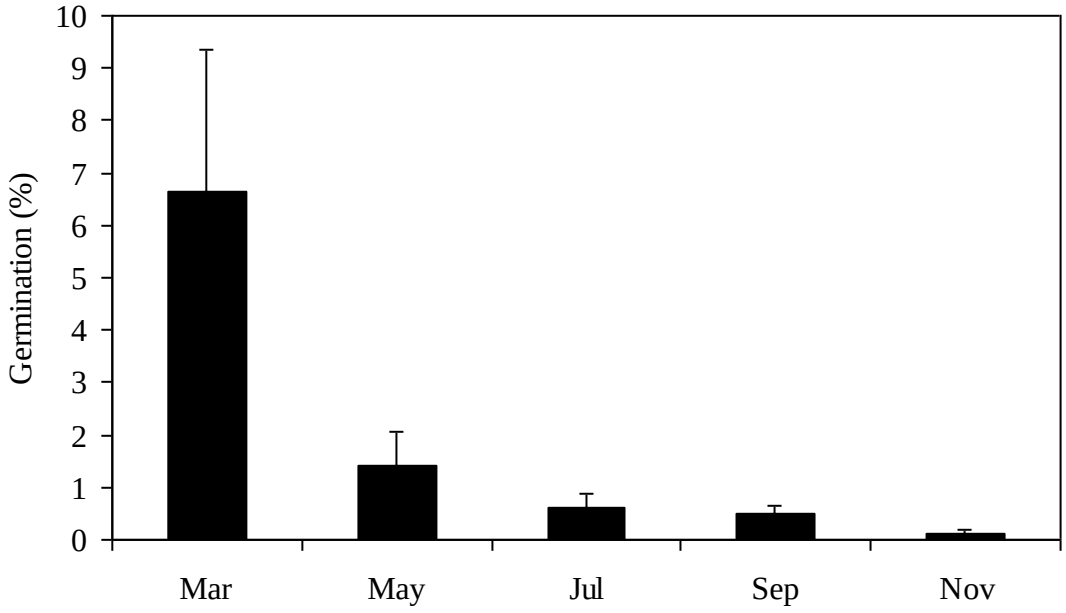


Figure 6.4: Percent germination of seed collected from plants harvested from March to November, 2005 (P: 0.003, LSD: 0.101). Error bars represent the standard error of the mean from five replicates.

*Natural movement experiment*

Approximately half of the plants (51%) did not travel further than 100 m from the release point, because they became entangled with other mobile or non-mobile *S. australis* plants in the stand (Figure 6.5, Figure 6.6). The plants that escaped the immediate population generally travelled until their movement was stopped by the fence surrounding the field, although 16% of plants traversed the fence to move into neighbouring fields (Figure 6.6). Approximately 10% of plants were found over 1 km from the release point, and the maximum distance travelled was 1247 m. The field to the east of the release point contained cattle, which crushed all plants that moved into this area. At the time of collection, 27 days after being released, all mobile plants were firmly anchored to other *S. australis* plants, fences or roadside vegetation. The six missing plants may have been hidden within the stand of plants at the release point, hidden from view if they were crushed by cattle or may have moved beyond the search area.

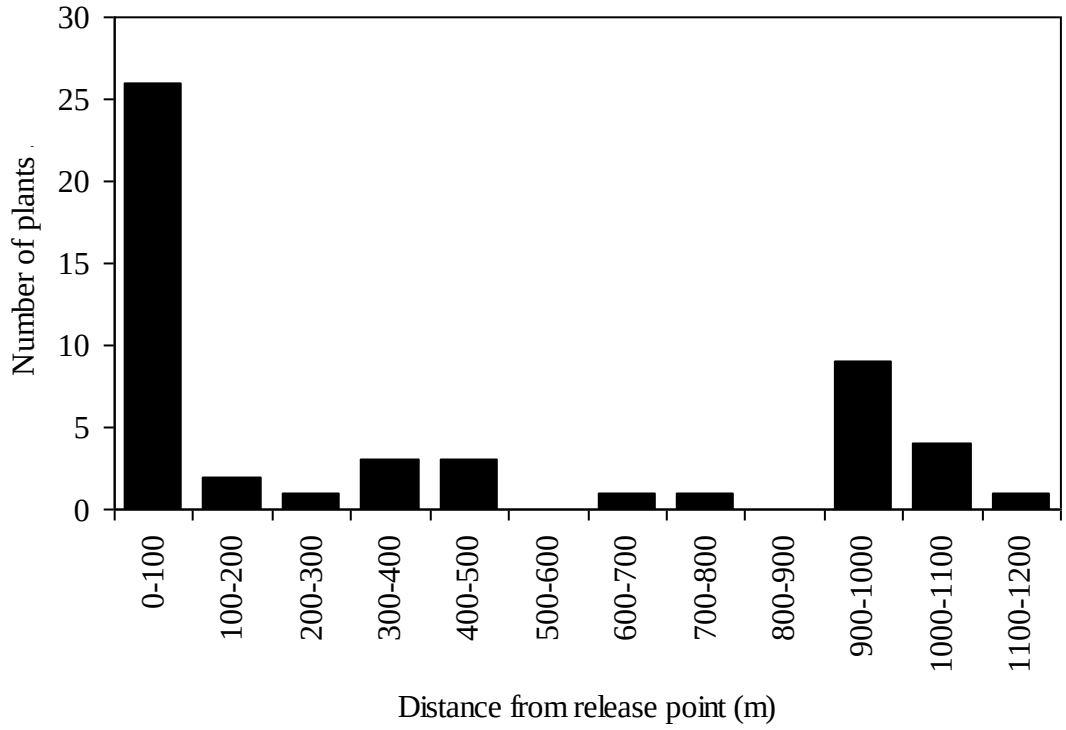


Figure 6.5: The number of plants found within a set radius (0-1200 m) from the release point in a field of wheat stubble, 27 days after they were released in the field.

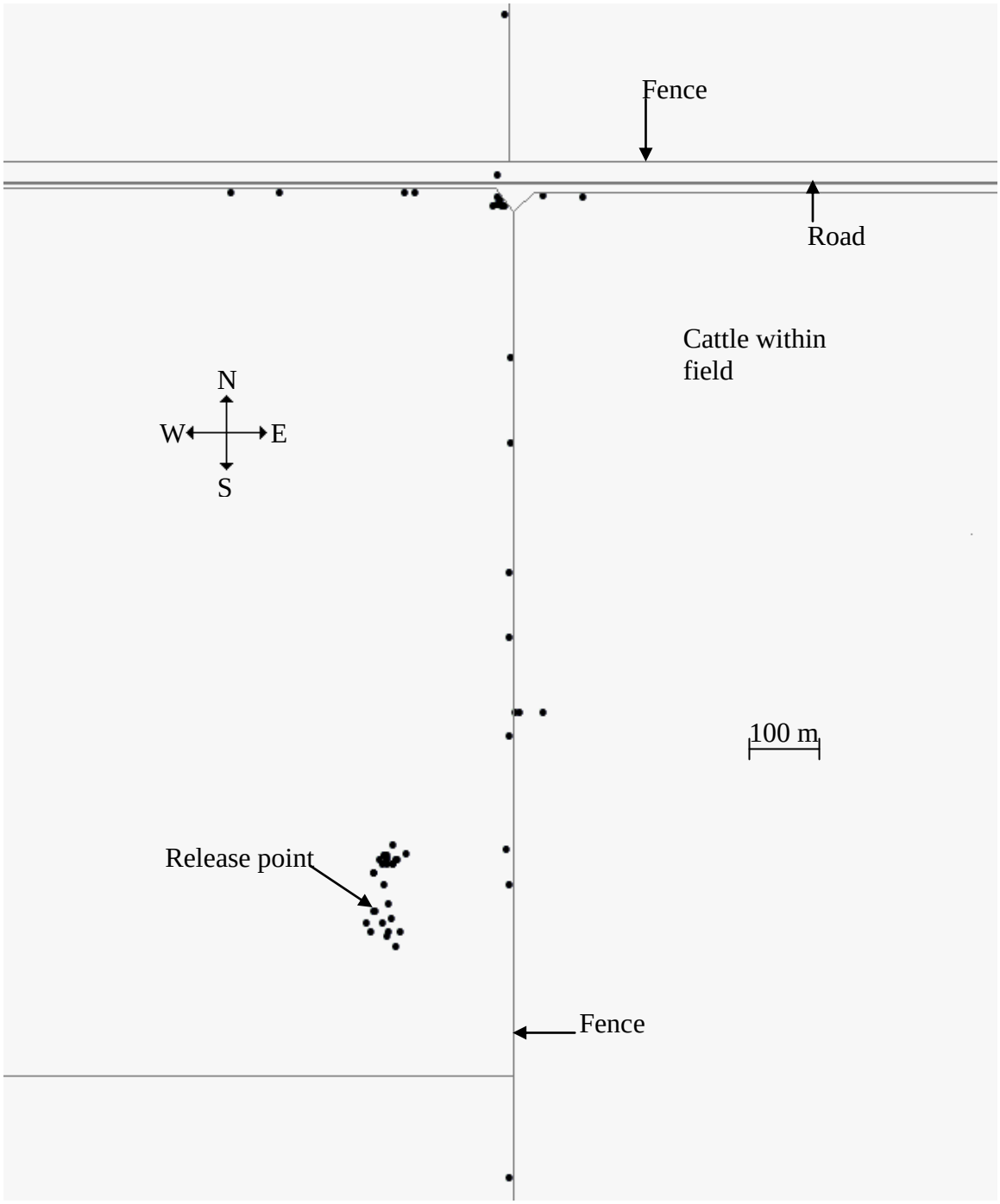


Figure 6.6: Map of the field in which the plants were released, showing the starting point from which all 57 plants were released (release point) and the final location of the 51 plants (black dots) that were retrieved 27 days later. The remaining 6 plants were lost.

The mean direction of travel was at an angle of 26.34 degrees, or roughly NNE of the release point. However, eight of the plants were retrieved from a SSW to ESE direction, so the direction of travel had two modes. This was confirmed by the probability test of randomness and Rayleigh test of uniformity, both of which indicated that the data were not distributed uniformly and so direction of travel was not random ($P < 0.001$). The Chi-square von Mises analysis indicated that the data was not unimodal, i.e. the majority of plants did not all travel in a single direction. The kernel density estimate indicated that the retrieval locations were distributed to form two distinct modes, i.e. the plants travelled in two different directions (Figure 6.7). The final position of plants from the release point was related to the prevailing winds (Figure 6.8). While wind blew at all angles during the experimental period, the modal wind direction was NNE, as were the majority of wind events with a speed higher than 30 km/hr. Thus, as expected, greatest plant movement occurred downwind of the prevailing wind direction. No explanation was found to account for the movement of some plants in a SSW to ESE direction. Movement in this direction was not explained by wind events or animal activity, and there were no notable features of the terrain (i.e. ground slope, density of the remaining *S. australis* plants etc.) to suggest that there would be less resistance to plant movement in this direction compared to any other direction from the release point. It is likely that chance wind events moved plants to this location where they subsequently became fixed and could not be moved by other wind events.

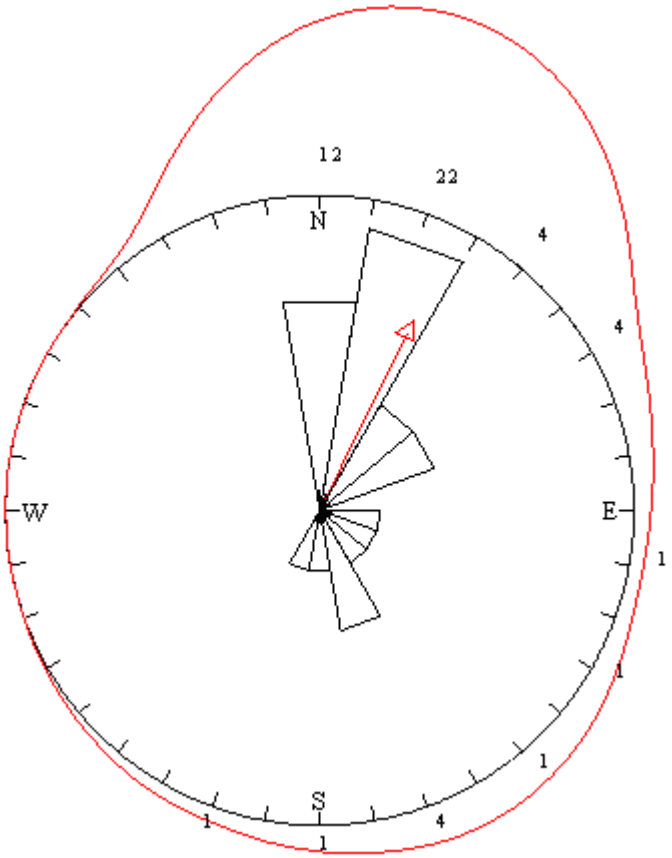


Figure 6.7: Direction, from the release point, of the retrieval location of each plant. Mean direction is indicated by the arrow. The numbers outside the circle indicate the number of plants that were retrieved at that direction from the release point. The outer line indicates the kernel density estimate (Bandwidth: 1, $P < 0.001$).

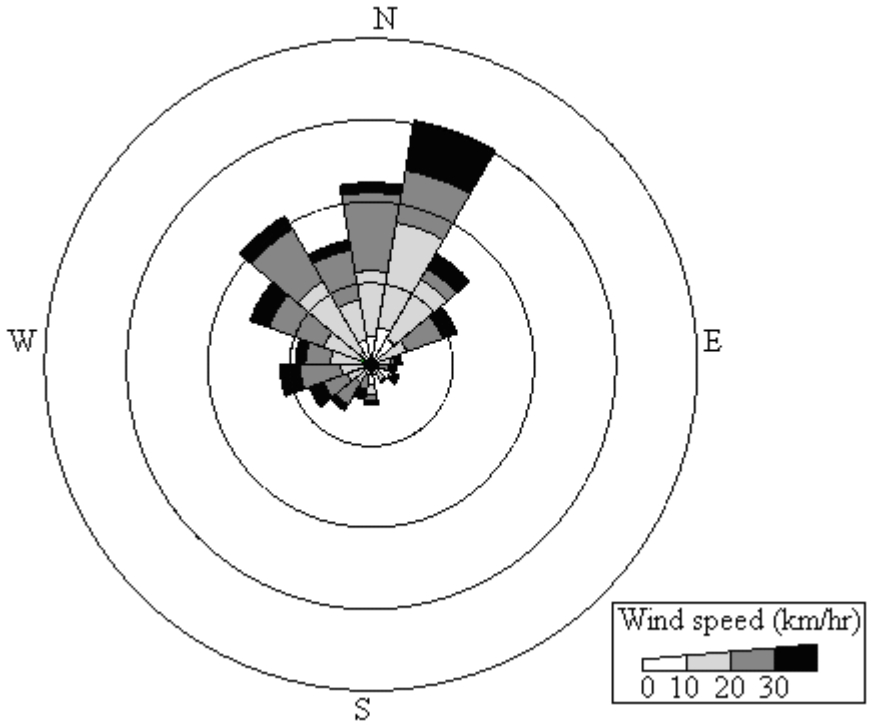


Figure 6.8: Maximum hourly wind speed from 7 April to 3 May 2006. The size of each segment corresponds to the number of wind events occurring in the given direction, and the shading indicates the speed of the wind events, according to the key.

There was no relationship between the apparent distance of travel from the release point and the amount of seed lost by plants (Figure 6.9). Seed lost per gram of plant material ranged from 16 to 67. Plants did not lose a significant amount of biomass through travel, since the final weight of plants was not significantly different to the starting weight. The seeds were too light for loss of seeds to affect plant biomass and the plants did not break up or easily lose branches as they moved.

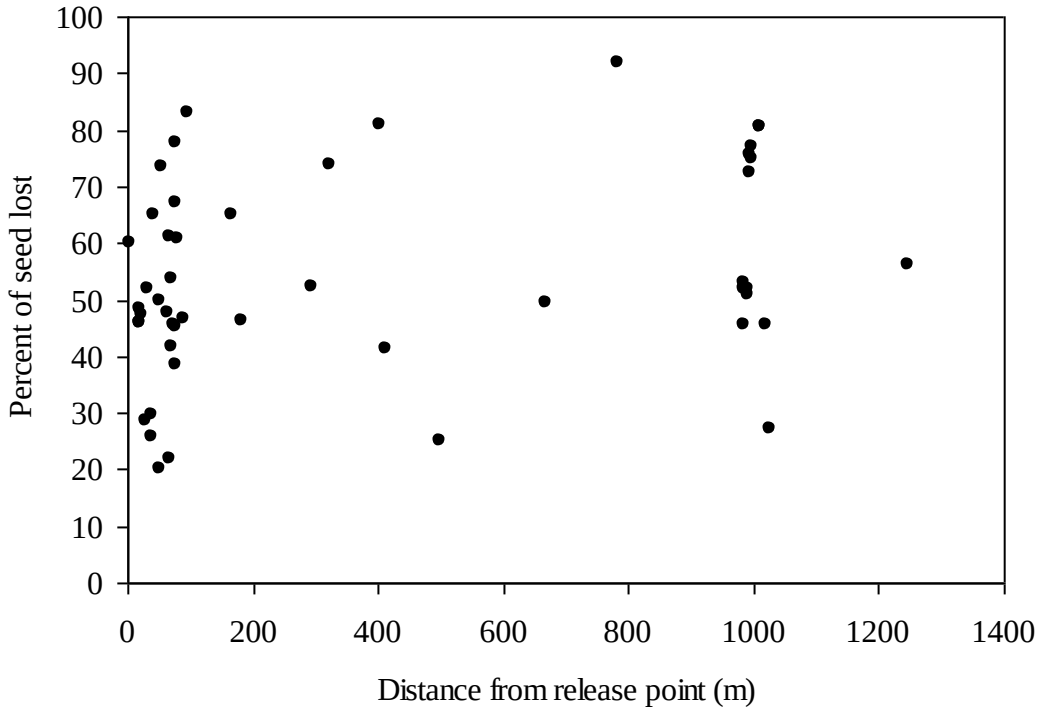


Figure 6.9: Percent of seed lost from each plant and distance of the plant from the release point, 27 days after the plants were released.

**Discussion**

Nearly a quarter (24%) of *S. australis* seed was shed by immobile mature plants, 58% of seed was shed from mobile plants and 17% of seed was retained on the plant regardless of plant movement or aging. Plants predominately moved in the direction of the prevailing winds, although no explanation was found to indicate why some plants moved in other directions. This indicates that *S. australis* plants moved and shed seed in a manner similar to that for *S. iberica* or *S. kali* (Mallory-Smith et al. 1993; Stallings et al. 1995; Young 1991). The only major difference appears to be that during travel, *S. australis* plants lost seed through shedding. The plant remained intact and seed retained on the plant did not enter the soil seed bank as did the shed seed. Mobile *S. iberica* or *S. kali* plants shed seed but also lost individual branches, allowing the proportion of seeds that remain attached to the branch to enter the soil seed bank as well as the shed seed. The similarity between these species is not surprising since it has been suggested that *S. iberica* is an incorrect name applied to *S. tragus* plants, and *S. australis* is morphologically similar to *S. tragus* and *S. kali* (Mosyakin 1996; Rilke 1999a) (Chapter 3).

The proportion of seed that is dispersed may be reduced in dense populations. This research found that other *S. australis* plants more effectively prevented movement than any other obstacles in the field (i.e. crop stubble, fences). Approximately half the test plants became attached to *S. australis* plants growing nearby and did not move beyond 100 m. The shedding of seed before dispersal ensures that some seed always remains near the mother plant. However, retention of mobile plants within a given patch (with the seed they carry) would ensure the maintenance of a larger seed bank, since seed retained on plants is shed through aging and weathering even if plants do not experience wind generated movement. The plants in this experiment were large and formed a dense population. Further research at other sites with plants of alternative size is required to determine if small plants in sparse populations are less likely to be retained in the habitat in which they are generated through entanglement with other *S. australis* plants. It is possible that agricultural environments favour growth to a greater degree than other ruderal environments (due to annual soil disturbance, fertiliser applications etc.), leading to an overabundance of plants and resulting in a hindrance of movement that would not occur in other ruderal habitats. Retention of *S. australis* plants at the site of origin ensures the maintenance of large populations and a constant supply of plants escaping to neighbouring fields and populations of *S. australis*. This maintains a high rate of invasion and range expansion (Nathan and Muller-Landau 2000).

Regardless of movement, all plants retained a proportion of their seed. The germinability of the retained seed was reduced from 6.6% to less than 2% over a matter of weeks. Variation in viability of fresh *S. australis* seed produced in field conditions ranges from 2-40%, and so initial germinability of 6.6% is not unusual (Chapter 5). Germinability of seed declined rapidly, but this may have little effect on total seed dispersal and seedling recruitment in the following season. Seed shedding was related to plant movement, and *S. australis* plants became firmly attached to obstacles and stopped moving in a matter of weeks. Any seed still retained on the plant once it stops moving is unlikely to shed, and so the germinability of this seed is no longer relevant. Loss of germinability for attached seed has not been tested over time for other species of the *Salsola* genus, but seed remaining attached to *S. kali* plants lost viability in less than a year (Young 1991). The retained portion of seeds would have very little impact on the population dynamics of this species in a natural environment. In an agricultural

environment where the plants are likely to be crushed through cultivation and incorporated into the soil, these retained seeds would contribute to seedling recruitment.

*Salsola australis* has two mechanisms for seed dispersal (diplochory), only one of which is considered in this paper. Seeds travel while attached to the plants, but continue to move once they have been shed. This work does not take into account post-shedding seed movement (i.e. by wind, granivores, or cultivation). This secondary dispersal is probably important in allowing seeds to move to an environment favourable for germination following long distance dispersal, i.e. cultivation buries seed, dispersing it to suitable recruitment microsites (although seeds of *Salsola* species are small and burial deeper than 3-7.5 cm can reduce successful establishment) (Wallace et al. 1968; Young and Evans 1979). However, secondary dispersal is unlikely to significantly increase total distance travelled (Cousens and Mortimer 1995; Vander Wall and Longland 2004). Wind dispersal of seeds is not a highly efficient form of dispersal even in species with aerodynamic seeds, and is not important compared to the hundreds to thousands of metres that the seeds of *S. australis* move while attached to the mobile plants (Cousens and Mortimer 1995; Sheldon and Burrows 1973). Seeds may travel further during extreme climatic conditions. The importance of very strong wind events on long distance seed dispersal in Western Australia has been demonstrated previously in *Banksia hookeriana* (He et al. 2004). However, the number of these seeds that successfully germinate and establish colonies (effective dispersal distance) is much lower, since not all seeds disperse to a favourable habitat (Cousens and Mortimer 1995). Overall, secondary dispersal events may not have much effect on total seedling recruitment of *S. australis* within an agricultural field.

In an agricultural system, the most effective way to prevent an established population of *S. australis* from increasing would probably be to stop disturbing the site through cultivation that occurs during the crop sowing operation. Cultivation in the cropping system does not control *S. australis* because it is a weed of the summer fallow and most seed production is completed before cultivation occurs. *Salsola australis*, an early successional and annual species, requires regular soil disturbance to form long lived populations. Thus reduced disturbance would allow the population to decline as occurs in natural habitats that have previously been disturbed but are not exposed to further disturbance (Smith and Read 1997; Wali 1999). Population decline in the absence of

disturbance (regardless of seed production) has been observed in several species of the genus *Salsola*, and possibly results from autotoxicity or an inability of the non-mycotrophic *Salsola* species to compete with mycorrhizal species (Schmidt and Reeves 1989; Smith and Read 1997). Reducing disturbance is impractical unless it is economically feasible to enter a pasture phase for two to three years where annual cultivation does not occur. Populations may also be prevented from expanding by removing or burning the mature plants. This would prevent the bulk of the seed from entering the soil seed bank. Since the dormant soil seed bank of agricultural weed species of the genus *Salsola* are short lived (i.e. 1-3 years) (Al-Charchafchi et al. 1987; Creager 1988; Evans and Young 1972; 1980), population growth is dependent on annual seed production. The normal practice of crushing plants through cultivation to allow the seeding operation to proceed at the end of autumn is the least effective management option, since this ensures that all seeds retained on the plants enter the soil seed bank.

Prevention of the establishment of new populations of *S. australis* on agricultural land may be more straightforward than reducing established populations, since the proportion of mobile plants that escape their own field are not likely to affect seedling recruitment in neighbouring fields. In this study approximately 25% of the plant population escaped the field after travelling close to 1000 m to reach the outer edges of the field. Once plants have travelled 1000 m, additional movement results in less than 10% of additional seed loss. Thus in the Western Australian wheat-belt where large fields dominate the landscape it is unlikely that plants that escape a field will shed a significant amount of seed in a new field as they move to their final resting place. Therefore, as long as these immigrating plants are removed (currently achieved by some farmers through burning or hay raking techniques) rather than being crushed to incorporate the seed into the soil, they have little chance of establishing a new population.

These trials were completed on single populations of *S. australis*. There is considerable morphological diversity evident between *Salsola* populations throughout Western Australia (Rilke 1999a). However, the populations examined here was the most common agricultural weed form that is found throughout Australia (Rilke 1999a) and populations of this form are genetically similar in Western Australia (Chapter 3). The

reproductive system of this species has not been examined, but morphologically similar species of the *Salsola* genus are out-crossing, wind pollinated species (Rilke 1999a; Young 1991). However, further research is required to determine if seed dispersal is uniform between *S. australis* populations in other regions of the Western Australian wheat-belt.

Management of this species will clearly require removal of mobile plants (and the seed they retain) that migrate into a field, although the number of mobile plants immigrating into a new field that are required to establish a new population warrants further investigation. Removal of mobile plants, as opposed to incorporating them into the soil, would reduce seedling establishment and population growth rate. Successful weed control will require further research into the management and reduction of the mobile seed bank.

# Chapter 7

# Survivorship and fecundity of the weed *Salsola australis* R.Br. in the agricultural region of southwest Australia


**Abstract**

The population dynamics of *Salsola australis*, a summer annual weed of broad acre cropping and pasture systems, were monitored on two farms in the Lake Grace district of Western Australia. Within an annual cropping system, weed control practices killed all *S. australis* plants in late autumn and winter, restricting *S. australis* to a summer annual growth habit. Within a pasture or fallow system, cohorts of *S. australis* seedlings established in almost every month of the 12 month assessment period and plants were not restricted to a summer annual growth habit. However, in all agricultural systems, the majority of seed was produced by cohorts present over summer. Estimated seed production ranged from 95 to 19 596 seeds per plant. Total seed production per year in a cropping system (12 584 seeds per $m^2$) was half that of non-cropped areas. Grazing by sheep in a pasture system did not significantly reduce total annual seed production (27 914 seeds per $m^2$) compared to that of the fallow system (39 854 seeds per $m^2$). Seed production of *S. australis* in a cropping system could be further reduced by control of summer cohorts.


## Introduction

Successful management of a weed species involves manipulation of the population dynamics to reduce the population size (Cousens and Mortimer 1995). This requires knowledge of the species life-cycle, seed dispersal processes, reproduction and mortality, and the factors that influence them. Many authors consider that the key to the formulation of successful life cycle models and development of weed management strategies is accurate data on weed biology, such as weed demography (Jordan 1992), seed germination and plant survival (Cousens 1995; Holst et al. 2007; Kropff et al. 1996). This principle has been applied to winter annual weeds that commonly occur in southwest Australian cropping systems, and the data on population ecology has subsequently been used to develop successful weed management strategies (Monjardino et al. 2003; Pandey and Medd 1991; Pannell et al. 2004).

In contrast to winter annual weed species, there is little information on the population dynamics and management of weed species of the summer fallow period in the Western Australian cropping system. However, summer fallow weeds such as *S. australis* in the wheat-belt of southwest Australia are of economic significance and require management (Mussell and Stewart 2004; Osten et al. 2006). Like other summer weeds, *S. australis* depletes stored soil moisture and nitrogen over the summer fallow period, reducing the yield potential of subsequent winter crops (Osten et al. 2006; Tennant 2000). The time taken to remove summer weeds in autumn (to prevent plants from blocking crop sowing equipment) delays crop sowing, which also leads to reduced crop yield potential (Tennant 2000). Within agricultural environments *S. australis* is a fire hazard and a general nuisance, with senesced, mobile plants damaging infrastructure such as fencing (Mussell and Stewart 2004; Smith 2004).

*Salsola australis* has been a weed of disturbed habitats in Australia for over 200 years (Beadle 1981; Rilke 1999a). It is found throughout Australia and is abundant in the Western Australian wheat-belt (Wilson 1984). Despite the abundance of this species, there is little or no literature on the impact of this weed in Western Australian agricultural systems. However, morphologically similar agricultural weed species of the *Salsola* genus have been shown to be detrimental in similar broad scale cropping systems in Washington State, USA, through competition with the crop and use of nutrients and stored soil moisture in fallow periods (Fowler and Hageman 1978; Schillinger and Young 2000; Young 1988). Beyond a species description (Mitchell and Wilcox 1988; Wilson 1984), there is no literature on the biology of this species, in Australian natural or agricultural systems. The objective of the work presented here is to describe the population ecology of *S. australis*, with emphasis on the establishment, survival and reproductive potential of populations in the Mediterranean agricultural system of southwest Australia. This information is essential in the development of effective management strategies for *S. australis* in Western Australian agricultural systems.

## Materials and methods

### *Trial site location and treatments*

Areas with *S. australis* populations were identified on two farms in the Lake Grace district on 13 December 2004. A site 20 m by 30 m was established on each farm, at S

33°14.507' E 118°26.960' on Site 1 and S 33°07.384' E 118°28.568' on Site 2. Site 1 had been sown to barley in the 2004 winter growing season (June to November) and contained barley stubble at the beginning of the trial period in December 2004. The field was left fallow over summer. In May 2005 the site was divided into two 10 m by 30 m plots, each with ten 0.25 $m^2$ quadrats. One plot was left fallow (fallow plot) and one plot was sown to barley (cropped plot). The cropped plot was treated with herbicides, including glyphosate (360 g a.i. $ha^{-1}$) presowing, metribuzin (112.5 g a.i. $ha^{-1}$) and trifluralin (400 g a.i. $ha^{-1}$) pre-emergence and 2, 4-D amine (500 g a.i. $ha^{-1}$) post-emergence. Site 2 (pasture plot) was cropped with wheat in 2004 and was a ley pasture that was periodically grazed by sheep during the 2005 growing season. Twenty 0.25 $m^2$ quadrats were established within the pasture plot.

Plant number and the growth stage of each plant within the quadrats in each plot were recorded once a month from December 2004 to November 2005. The height and width of each plant was recorded at senescence and used to estimate seed production (Figure 7.1). The plants were almost spherical, and width was measured at the widest point. The month in which the plants became mobile was recorded by noting when senesced mature plants were no longer in the quadrat. Plants that died before reaching the reproductive stage were more likely to degrade in place rather than become mobile, but those that did become mobile were not recorded as mobile plants since they carried no seed and thus did not impact on population dynamics.

*Data analysis*

ANOVA was used to assess the effect of each agricultural practice (i.e. crop, pasture and fallow plots) and time of the year on plant density. Differences between means were tested using least significant differences ($P < 0.05$) (GENSTAT Version 8.2). Average plant width and length of the life span for those plants that completed their life cycle (establishment to senescence) was compared between sites using t-tests. Average seed production per metre per year was also compared using t-tests ($P < 0.05$).

*Estimation of seed production*

To determine the relationship between seed production and plant size, plants were collected twice (in February and March 2005) from areas adjacent to the trial sites and their height and width were measured. Plants were oven dried at 40°C for one week

before weighing and threshing. Seeds were counted in five sub-samples of 5 g of dry plant material. The average seed number per gram of biomass and the total plant biomass was used to estimate total seed number per plant. The relationship between plant height, width and seed number per plant was investigated through regression analysis (GENSTAT Version 8.2). Total seed number (transformed to a $\log_{10}$ scale) was linearly related to plant width (Figure 7.1). The relationship was not significantly different between each site and was not significantly improved by incorporating plant height or volume.

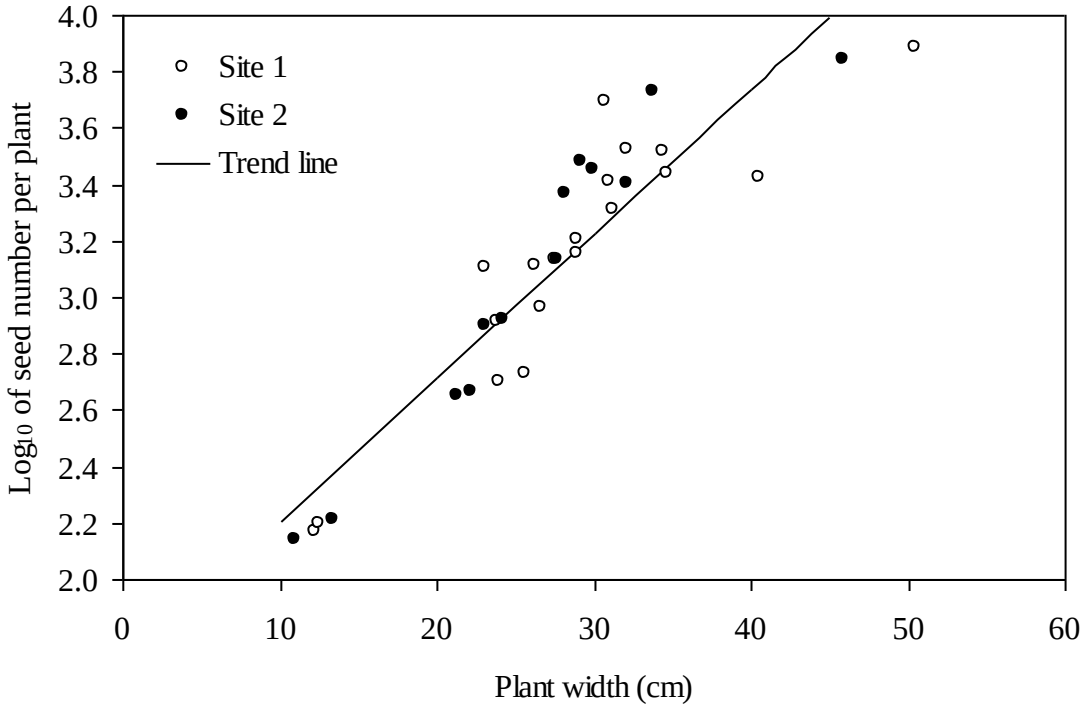


Figure 7.1: Seed produced per plant ($\log_{10}$ transformed data) and plant width (cm), for plants collected from near Site 1 and 2. The trend line indicates the linear relationship identified by the regression analysis ($y = 1.696 + 0.051x$, $r^2 = 83.6\%$, $P_{0.05} < 0.001$).

This relationship was used to estimate seed production of plants within plots, using the plant width data collected at plant maturity. The relationship was determined using plants that senesced in February and March (summer/autumn) 2005, because the majority of plants within the plots for which seed production was estimated had senesced and become mobile at this time. However, some plants within the plots matured in winter and spring, and the same relationship was used to estimate their seed

production. It was assumed that the relationship between seed production and plant width would remain constant for plants maturing at all times of the year.

*Climate*

Climate data was obtained from the Lake Grace Bureau of Meteorology weather station, located approximately 14.92 km from Site 1 and 3.48 km from Site 2. The climate at Lake Grace from December 2004 to November 2005 was similar to the long term average climate for this region (calculated from 1914 to 2006 for rainfall and 1956 to 2006 for temperature). The minimum temperatures from July to November 2005 were all approximately 1$^{o}$C lower than the average minimum temperature for these months, as were the maximum temperatures from September to November 2005 (Figure 7.2). Rainfall from December 2004 to November 2005 was 381.2 mm, slightly higher than the average annual rainfall of 354.8 mm. However, summer rainfall (December 2004 to January 2005) was 24 mm, less than half that of the long term average summer rainfall of 52.2 mm. Summer rainfall in Lake Grace is very erratic, and from 1914 to 2006, has varied from 2.2 to 329 mm.

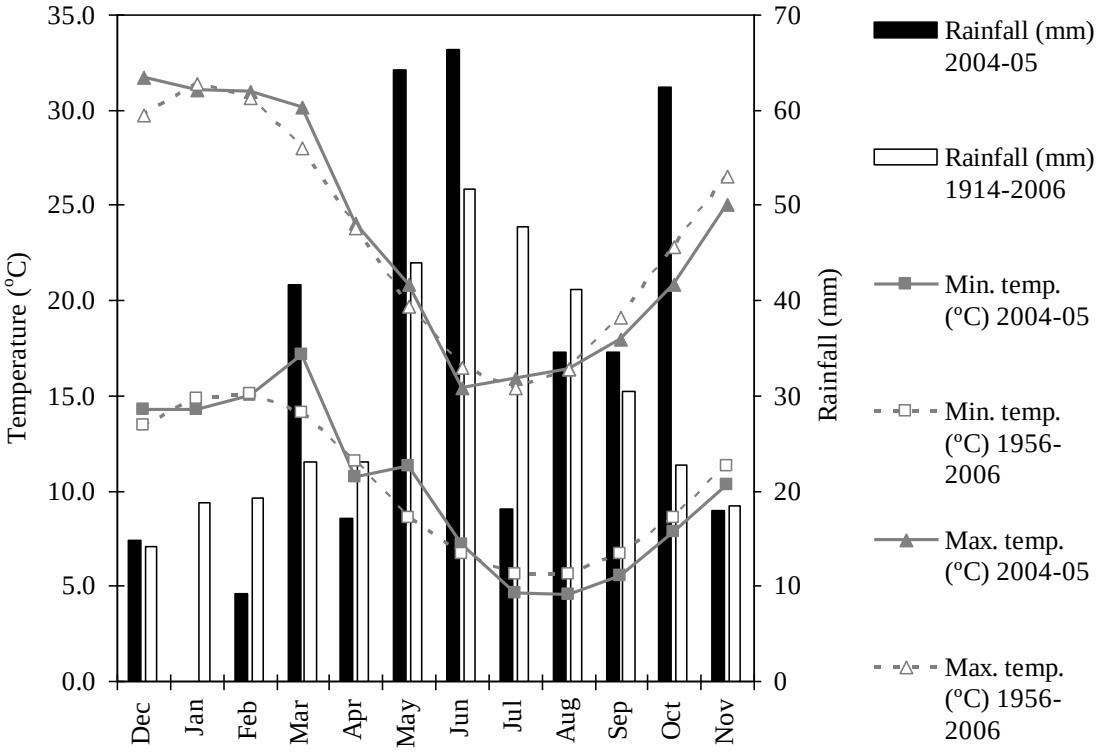


Figure 7.2: Average monthly maximum and minimum temperature and total rainfall over the trial period (December 2004 to November 2005), long term average maximum and minimum temperature (from 1956 to 2006) and long term rainfall (from 1914 to 2006). Climate data was provided by the Bureau of Meteorology from the Lake Grace weather station.

**Results**

*Plant growth stages*

Six stages in the life cycle of *S. australis* plants were identified; seedling, juvenile and adult vegetative growth stages, reproductive stage, above ground seed bank (seeds attached to senesced or mobile plants) and soil seed bank (seeds shed from the mobile plants). The seedling stage was defined as the emergence of cotyledons and establishment of a radical (Figure 7.3 A). The juvenile and adult vegetative stages could be differentiated by the shape of the leaves. During the juvenile vegetative stage, the leaves were round, soft and approximately 50 mm long (Figure 7.3 B). The adult stage of vegetative growth was characterised by leaves that were short (usually less than 10 mm), flat and tapered to a spine (1-5 mm long) (Figure 7.3 C). The reproductive stage was defined as the onset of flowering and seed production, and could commence immediately upon entering the adult vegetative stage or up to six months later (Figure 7.3 E). Adult vegetative growth continued during the reproductive stage. Some plants

produced juvenile growth from near the stem base during the adult vegetative growth or reproductive stages (Figure 7.3 D). The new branches in the juvenile vegetative stage then progressed through subsequent growth stages to reproduction and senescence. Once the entire plant had senesced, including any new branches that had sprouted, the above ground section of the plant was released from the root system to become mobile (Figure 7.3 F, G). These mobile, senesced plants formed a mobile seed bank. Seeds shed from senesced plants before or after they became mobile, forming a soil seed bank. The mature seeds (retained on the plant or shed) were contained in a fruiting perianth, which either had five papery wings or were wingless (Figure 7.3 H).

All plants that had reached the reproductive stage, and subsequently senesced, became mobile. Plants that died in the first stage of vegetative growth always degraded without being released from their root system. Plants in the adult stage of vegetative growth that died before reaching the reproductive stage were more likely to become mobile than to degrade while attached to their roots.

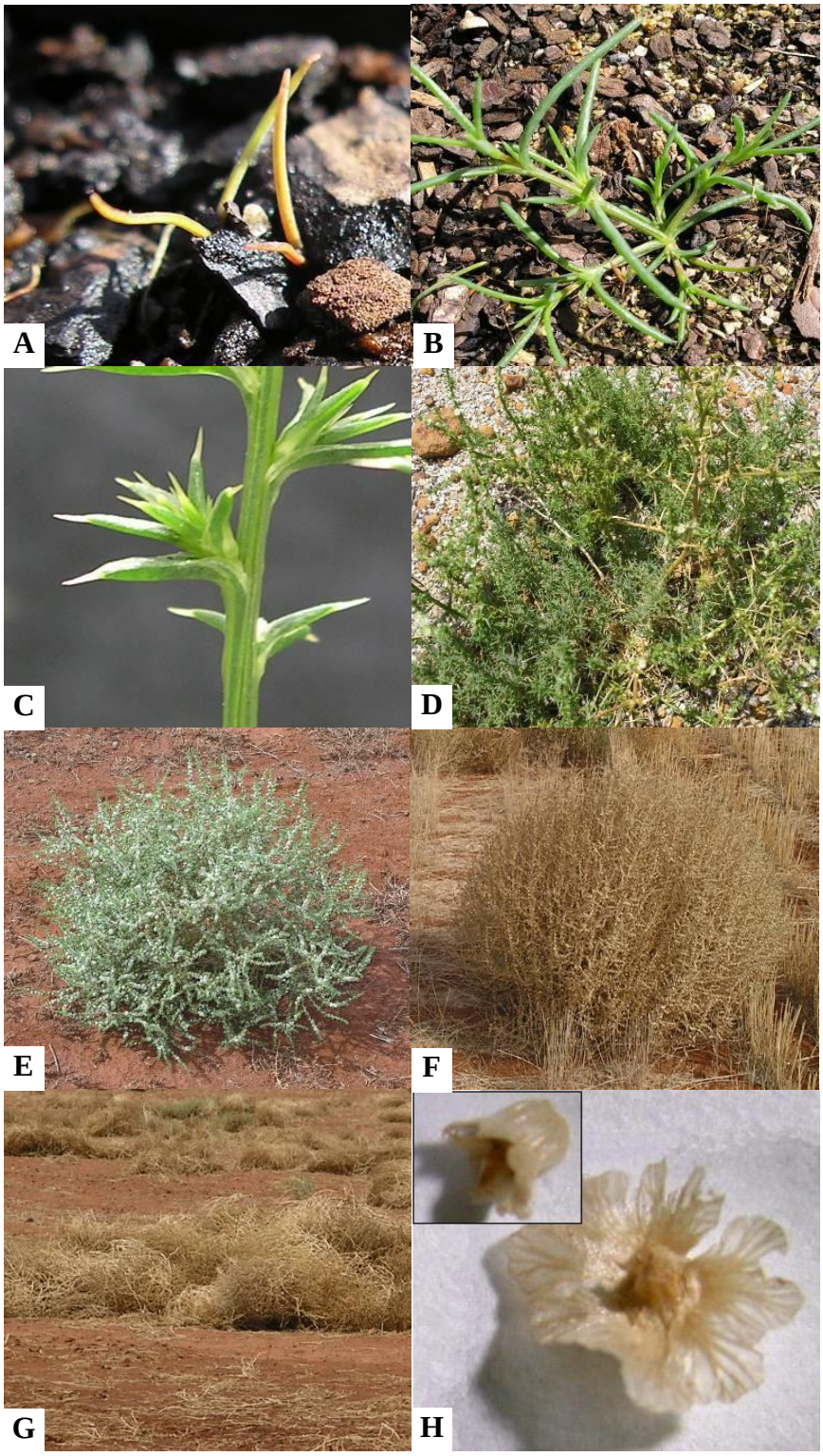


Figure 7.3: *Salsola australis* at various stages of the life cycle. Photos were taken from plants in the Lake Grace district in 2004 and 2005. Stages of the life cycle pictured above include: fruiting perianth with or without wings (containing seed) (A), seedlings (B), juvenile vegetative growth stage (C), adult vegetative growth stage (D), combined juvenile and adult vegetative growth stage (E), reproductive stage (F), senesced plant with viable seed (G) and mobile plants with viable seed (H).

*Annual effect of agricultural practices*

*Salsola australis* plants established and grew throughout the year in the pasture and fallow plots, with plants in all life cycle stages present at almost all times of the year. Within the cropped plot, plants grew throughout summer, but the crop sowing operation and the application of herbicides during May (autumn) killed all *S. australis* plants and further establishment did not occur until August. This did not affect the annual cumulative seedling emergence in the cropped plot compared to the other plots (Table 7.1), with seedling emergence and survival similar in the fallow and crop plots. The pasture plot had a greater density of seedlings but also a lower seedling survival (transition probability). As a result, annual cumulative density of plants in the vegetative stage was similar for all plots.

Of the plants that reached the reproductive stage, 54 to 83% had done so during summer and autumn in all plots. As a result, removal of plants by the crop sowing operation in late autumn reduced the total number of *S. australis* plants completing reproduction and reaching senescence. This reduced the average annual seed production in the cropped plot compared to the other two plots (P: 0.023, 0.032). By comparison, the pasture and fallow plots had similar numbers of senesced plants per $m^2$, and total seed production was similar between these plots. So cropping had a significant impact on the *S. australis* population when compared to the fallow plot, but grazing by sheep in the pasture plot did not.

Table 7.1: Average annual number of plants in each life stage (with standard error) for each plot, the probability of transition between life cycle stages and number of seeds produced for each plot (estimated from plant width).

| Plot | Stage | Number of plants $m^{-2}$ $yr^{-1}$ | Transition probabilities | Number of seeds $m^{-2}$ $yr^{-1}$ |
|---|---|---|---|---|
| Fallow | Seedling | 92.4 (1.9) | 0.96 | |
| | Vegetative stage | 88.4 (2.5) | 0.20 | |
| | Reproductive stage | 17.8 (0.3) | 0.98 | |
| | Senescence | 17.4 (0.5) | 0 | 39 854 |
| Crop | Seedling | 94.4 (2.3) | 0.90 | |
| | Vegetative stage | 85.0 (2.1) | 0.28 | |
| | Reproductive stage | 23.7 (0.7) | 0.39 | |
| | Senescence | 9.2 (0.4) | 0 | 12 584 |
| Pasture | Seedling | 146.0 (2.5) | 0.58 | |
| | Vegetative stage | 84.4 (1.4) | 0.29 | |
| | Reproductive stage | 24.3 (0.6) | 0.81 | |
| | Senescence | 19.7 (0.3) | 0 | 27 914 |

*Survivorship*

Cohorts of seedlings established in almost every month of the 12 month assessment period, resulting in overlapping generations of plants in various life cycle stages. At the start of the trial in December 2004, there were seedlings that had established that month and plants that had established prior to December at each site. Successive cohorts appeared in each of the following months of the trial period (Figure 7.4). Within each single cohort, plants experienced uneven progression through growth stages. Each cohort declined as plants died prior to reproduction or through senescence following the reproductive stage. However, within the cropped plot all cohorts were removed in May and further establishment prevented until August. Obvious causes of mortality from invertebrate herbivores or pathogens were not observed.

The number of seedlings that established in December ranged from 4 to 18 plants per $m^2$ greater than the number of seedlings that established in the January or February

cohorts. This was most likely due to the increased rainfall received in December when compared to that received in January or February (Figure 7.2). There was no rainfall in January and the seedlings that established during this month must have germinated in response to stored soil moisture resulting from the December rainfall. The largest cohorts established in autumn and winter. The large winter cohorts established at a density of 36 plants $m^{-2}$ in the crop plot in August, 27 and 26 plants $m^{-2}$ in the fallow plot in April and May, and 22 plants $m^{-2}$ in the pasture plot in April.

Mortality rate of *S. australis* cohorts was dependent on the time of cohort establishment. Survivorship curves could be classified as type I, type II or type III, according to the commonly used classification system discussed by Deevey (1947). Survivorship of the December cohorts appeared to decline gradually in the crop plot (until removed by the cropping enterprise) (Figure 7.5 A). The December cohorts in the fallow plot and the December and February cohorts in the pasture plot declined at a constant rate as they aged throughout the year (i.e. type II survivorship curves, Figure 7.5 B, C). The February cohort in the crop and fallow plots, and the January cohort in the pasture plot, declined very rapidly in the months following cohort establishment (type III). However, the January cohort in the fallow plot declined gradually until September, when all plants died (type I). Within the fallow plot, the April cohort declined rapidly, as did the September cohort in the crop plot. The other autumn, winter and spring cohorts in all three plots declined gradually throughout the year (type I).

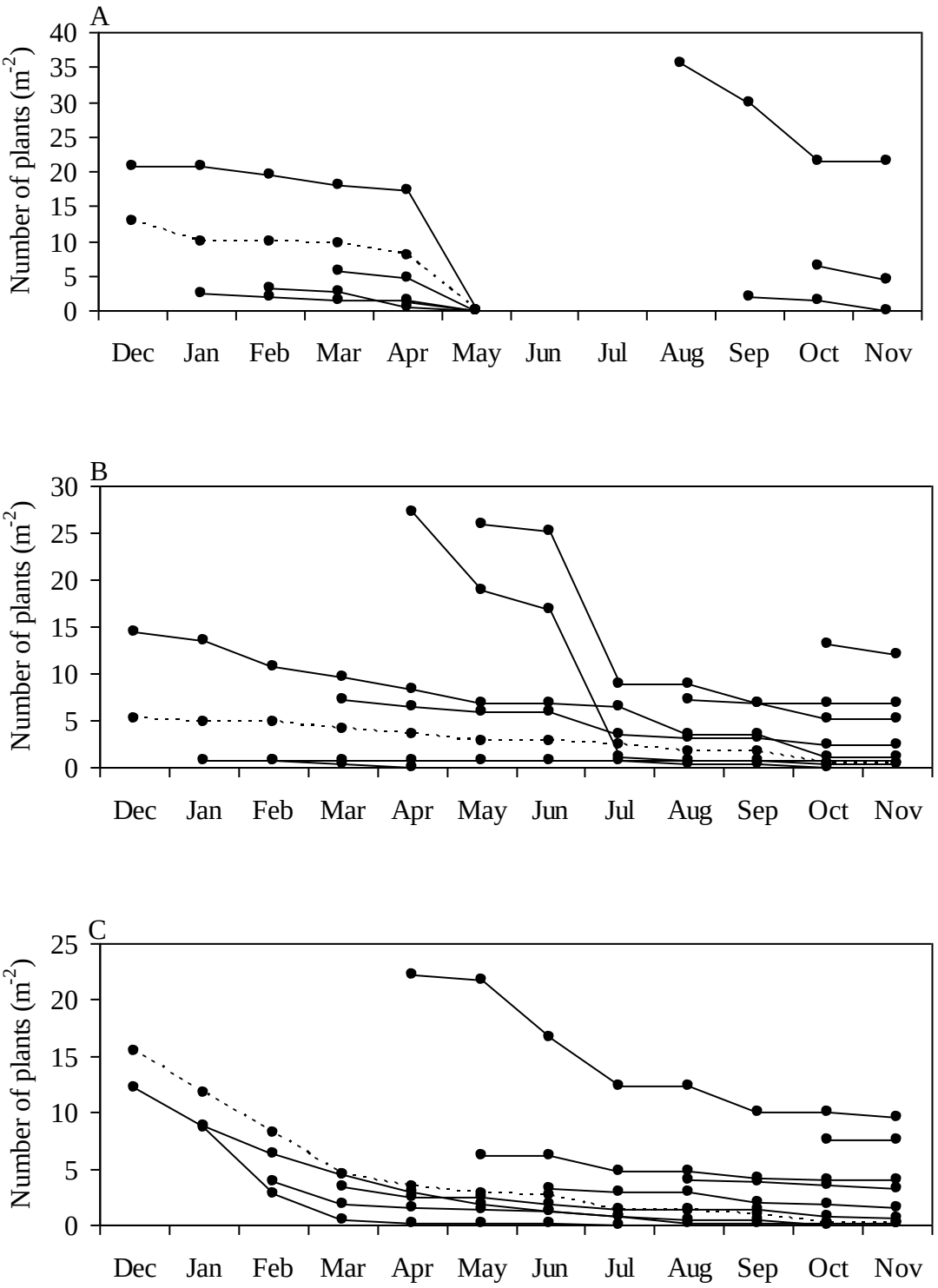


Figure 7.4: Initial number of plants in cohorts appearing in each month and number of surviving plants as cohorts age during successive months (from December 2004 to November 2005) as average number of plants per $m^2$, at the crop (A), fallow (B) and pasture (C) plots. Solid lines indicate cohorts of seedlings appearing each month. The dotted line indicates the plants that were present in December that had established prior to the commencement of the trial period.

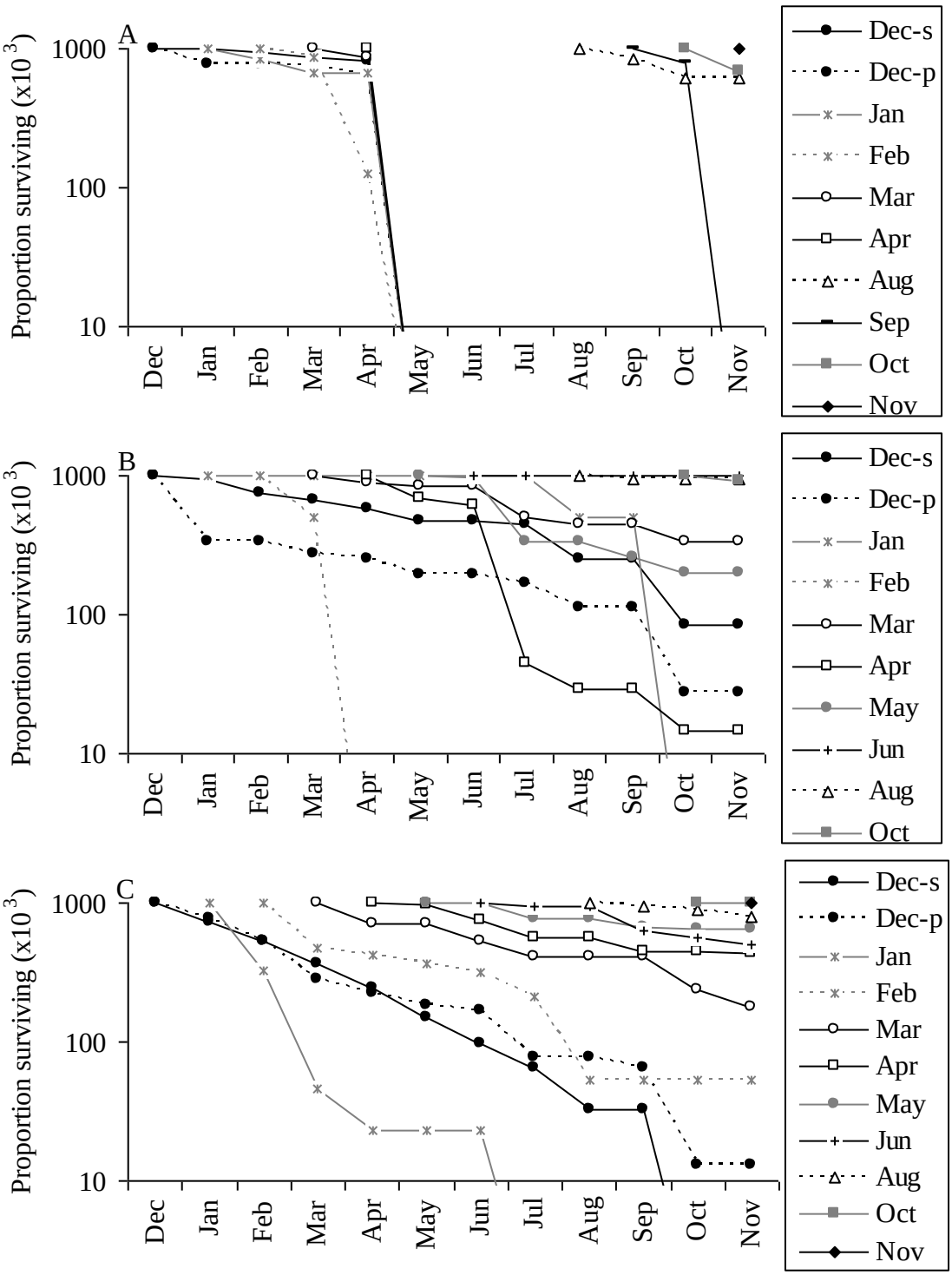


Figure 7.5: Survivorship curves of plants in monthly cohorts, at the crop (A), fallow (B) and pasture (C) plots. Each cohort is listed in the legend, with Dec-s and Dec-p indicating seedlings or plants present in December. The number of survivors in each cohort (as a proportion of the original cohort size) is plotted on a $\log_{10}$ scale, to show rate of mortality.

*Plant density*

Total plant density was significantly affected by the cropping operation in the crop plot and by climate in the fallow plot (Table 7.2). Plant density in the crop plot was not significantly different throughout the year, except from May to July where it was reduced to zero. In the fallow plot, plant density was significantly higher during May to July, in the months of highest rainfall, and lower in January to March, in the months of low rainfall (Figure 7.1). Plant density in the pasture plot was uniform throughout the year. Plant density in all plots remained constantly high as seedling cohorts were present in every month.

Table 7.2: The average plant density (with standard error) in each plot (crop, fallow and pasture) at each measurement period from December 2004 to November 2005 ($P < 0.001$, LSD: 14.16).

| Month | Plant density (plants $m^{-2}$) | | |
|---|---|---|---|
| | Crop | Fallow | Pasture |
| Dec | 33.6 (4.3) | 19.6 (2.8) | 27.6 (3.4) |
| Jan | 33.2 (4.3) | 19.2 (3.3) | 29.0 (2.5) |
| Feb | 34.8 (3.5) | 17.2 (2.3) | 21.0 (1.2) |
| Mar | 37.6 (3.2) | 22.4 (1.9) | 14.2 (0.8) |
| Apr | 33.3 (2.7) | 46.4 (4.8) | 32.7 (3.9) |
| May | 0.0 (0.0) | 60.8 (8.1) | 36.6 (5.2) |
| Jun | 0.0 (0.0) | 59.2 (8.9) | 32.9 (4.5) |
| Jul | 0.0 (0.0) | 24.0 (1.8) | 24.4 (4.0) |
| Aug | 35.6 (7.1) | 26.4 (1.8) | 27.4 (3.8) |
| Sep | 31.8 (5.9) | 23.8 (2.6) | 23.0 (3.8) |
| Oct | 38.2 (6.2) | 30.4 (3.1) | 28.2 (3.8) |
| Nov | 30.4 (5.1) | 29.2 (5.0) | 27.2 (5.0) |

*Seed production per cohort*

The plants that established prior to or during December 2004 in all three plots produced more seed than any of the other cohorts that established later in the year (Table 7.3). The percentage of total seed production per year by plants or seedlings present in December ranged from 52.71% in the fallow plot to 100% in the cropped plot. The cohorts that established in autumn, winter and spring produced very little seed before

the trial ceased at the end of November 2005. However, plants from these cohorts likely produced seed in the following year, as plants in several cohorts were still alive, but had not yet reached the reproductive stage by this time (Figure 7.4).

Table 7.3: Total and percentage seed production over the 12 month period of the trial for each plot (crop, fallow and pasture) from each cohort indicated in Figure 7.4. The percentage is calculated from the total seed production in each plot over the trial period.

| Month | Crop | | Fallow | | Pasture | |
|---|---|---|---|---|---|---|
| | Total (seeds $m^{-2}$) | Percentage | Total (seeds $m^{-2}$) | Percentage | Total (seeds $m^{-2}$) | Percentage |
| Dec – plants | 1050 | 32.5 | 1839 | 18.5 | 2278 | 32.7 |
| Dec – seedlings | 2187 | 67.5 | 3413 | 34.5 | 2503 | 35.9 |
| Jan | 0 | 0 | 169 | 1.7 | 1001 | 14.3 |
| Feb | 0 | 0 | 0 | 0 | 717 | 10.3 |
| Mar | 0 | 0 | 2405 | 24.1 | 265 | 3.8 |
| Apr | 0 | 0 | 347 | 3.5 | 44 | 0.6 |
| May | 0 | 0 | 843 | 8.5 | 86 | 1.2 |
| Jun | 0 | 0 | 0 | 0 | 84 | 1.2 |
| Jul | 0 | 0 | 0 | 0 | 0 | 0 |
| Aug | 0 | 0 | 948 | 9.5 | 0 | 0 |
| Sep | 0 | 0 | 0 | 0 | 0 | 0 |
| Oct | 0 | 0 | 0 | 0 | 0 | 0 |
| Nov | 0 | 0 | 0 | 0 | 0 | 0 |

*Life span and seed production*

Plants that completed their life cycle (seedling establishment to mobility) during the 12 month study period had life spans ranging from 2 to 12 months, with estimated seed production of between 95 and 19 596 seeds per plant. However, there was no correlation between life span and plant width (or life span and seed production per plant estimated from plant width, $r^2 = 0.015$, P: 0.783). Average life span for plants in the cropped plot was 4.6 months. This was significantly shorter than that of the fallow or pasture plots where plants had an average life span of 7.2 and 5.3 months ($P < 0.001$). The shorter life span of plants in the crop plot resulted from the cropping enterprise (Figure 7.4 A). The cropping enterprise did not affect plants that reached senescence prior to crop sowing,

but did remove plants that would have continued to grow through winter and spring. However, in spite of the variable life span of plants, the average plant width in each plot (23.8 cm in the crop plot, 26.6 cm in the pasture plot and 27.8 cm in the fallow plot) was not significantly different (P: 0.074, 0.203). As a result, the average estimated seed production per plant did not vary between plots.

*Dispersal*

The number of plants reaching mobility was highly variable between months (Figure 7.6), and was affected by land use, plant density and climate. Plants did not reach senescence during December 2004 and January 2005, as the plants present in early summer were immature. In the cropped plot, plants became mobile from February to April. Some plants that became mobile in April were artificially released (and subsequently crushed) by cultivation during the crop sowing process. All other plants in the cropped plot were killed, ensuring that no plants reached senescence and mobility later in the year. In the pasture and fallow plots, plants became mobile during February to November. Within the pasture plot, the density of plants becoming mobile in any single month was never greater than 2 plants $m^{-2}$, and so was similar for each month in which plants became mobile. However, total plant density in the pasture plot was also similar between months. The number of plants becoming mobile in the fallow plot increased from an average of less than 1 plant $m^{-2}$ in February to 7 plants $m^{-2}$ in September, and then reduced to 3.5 plants $m^{-2}$ in November. Total plant density in the fallow plot also increased from February to June, and then gradually decreased (Table 7.2). There was no correlation between number of plants reaching senescence per metre per month and plant size.

Mobility appeared to be related to rainfall (Figure 7.2). Rainfall was greatest in May, June and October (>60 mm), when few plants became mobile. July and November had low rainfall (<20 mm), and plants became mobile at both the pasture and fallow plots during these months. However, plants became mobile during September, and both September and August experienced moderate rainfall levels (30-50 mm).

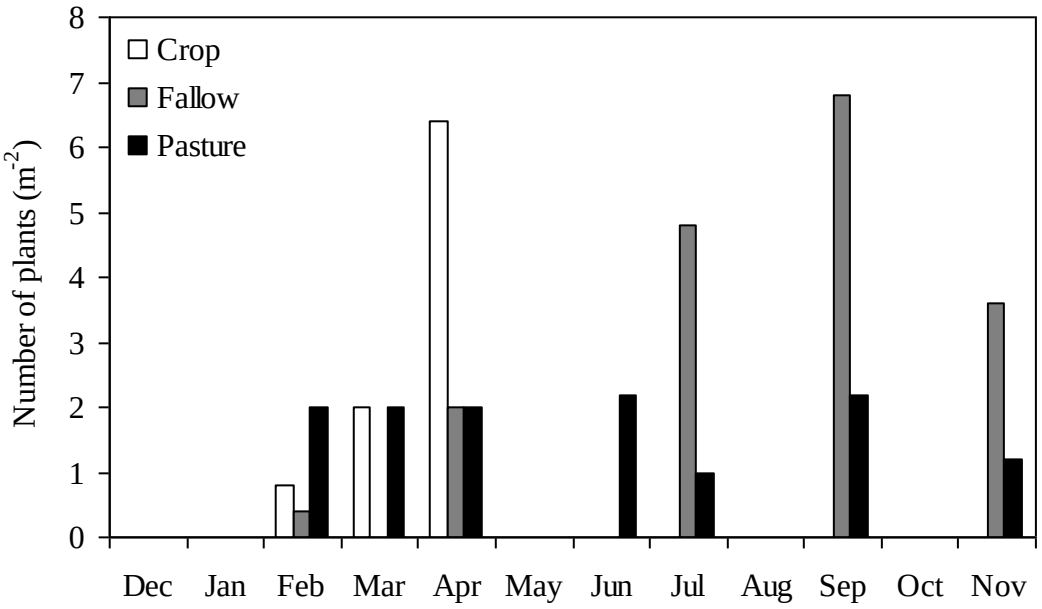


Figure 7.6: Number of plants ($m^{-2}$) becoming mobile in each plot (crop, fallow and pasture) during each month, from December 2004 to November 2005.

**Discussion**

*Salsola australis* has previously been considered as a summer annual weed of dry-land agricultural regions. However, this research indicates that in southwest Australia *S. australis* is restricted to a summer annual growth habit by the winter cropping system. Cropping did not affect the reproductive capacity of plants growing in summer, but killed *S. australis* plants present in autumn. All seed production of *S. australis* within the cropping system resulted from December cohorts of plants and seedlings, and so seed production in the trial year would have been further reduced by herbicide application to kill all plants in December. However, precipitation was higher in December than January or February. Summer cohorts declined rapidly and produced little seed when precipitation was low. So summer precipitation probably has a significant effect on both survivorship and total fecundity of the population. Summer rainfall is highly erratic in Lake Grace (Bureau of Meteorology 2007b) and the year of the trial period had below average summer rainfall, which may have resulted in below average seed production. In years with greater summer rainfall, total fecundity in all summer months may be significantly higher. Summer weeds like *S. australis* may become an increasingly severe problem, as increased summer rainfall is predicted for much of Australia as a result of climate change (CSIRO 2001; IPCC 2001).

In similar cropping systems in Washington State, USA, *S. iberica* is a highly competitive weed in spring and summer growing wheat crops where it germinates at and prior to crop establishment, but is less competitive in winter wheat where it germinates after crop establishment (Young 1986; 1988). *Salsola australis* germinates during and prior to crop establishment in Western Australia, but is not competitive during early crop growth. The low impact of *S. australis* may result from effective in-crop weed control during early stages of crop growth. Alternatively, the low winter temperatures may reduce competitive ability of *S. australis*. Failure of this weed to establish within the crop until August ensures that the crop can out-compete *S. australis* plants, and prevents seed production until the following summer (after crop harvest).

Compared to cropping, grazing in the pasture plot had very little impact on the population. Grazing of the pasture plot may have been responsible for the lower transition probability for seedlings, since sheep will eat the young plants (Jacob et al. 1992). However, seedling mortality in the pasture plot did not affect the number of plants in the vegetative and reproductive stage. Sheep do not eat the plants once they enter the adult vegetative growth stage, and so grazing did not affect total seed production. However, there is some evidence that populations of *S. australis* do not remain in pasture systems for more than three years due to inadequate levels of soil disturbance in the pasture regime (Dr Lindsey Bell, CSIRO, pers. comm.). This tendency for the population to decline in the absence of major disturbance (cultivation) in spite of annual seed production has been observed in other species of the *Salsola* genus, and may result from autotoxicity or an increase of fungal pathogens (Allen 1989; Allen and Allen 1988; Lodhi 1979; Schmidt and Reeves 1989; Wali 1999).

Within the pasture or fallow system, *S. australis* was not restricted to a summer annual growth habit. *Salsola australis* cohorts established throughout the year, although the rate of cohort decline (i.e. rate at which plants within the cohort died) was affected by time of establishment. Cohorts that established in December declined at a constant rate (type II), likely due to age-dependant mortality following reproduction as well as other mortality factors such as low summer rainfall or grazing. Most autumn, winter and spring cohorts declined gradually. Plants in these cohorts produced very little seed during the trial period, and so mortality was not age dependant. These cohorts would presumably decline rapidly upon reaching summer through senescence following

reproduction (type I). At both sites, the January and February cohorts experienced high initial mortality (type III). However, rainfall was very low during these two months and competition for limited water likely resulted in density dependent mortality. For annual species, cohorts usually decline in a type I or type II survivorship curve (Silvertown 1987). However, the production of cohorts that decline according to each of the three survivorship curves, depending on the time of cohort establishment, has been observed for other summer annual weeds. The agricultural weed *Tribulus terrestris* L. produced cohorts with survivorship curves corresponding to all three types (Verdu and Mas 2006). Type III survivorship curves were apparent for seedlings that emerged when soil moisture levels were reduced due to low precipitation.

Within the cropping system, all reproduction resulted from the December cohorts, but in the pasture and fallow plots, reproduction occurred throughout much of the year. The optimal time for *S. australis* plants to enter the reproductive phase clearly depended on environmental factors rather than plant age. Plants could remain in the vegetative stage for weeks or several months before entering the reproductive stage. The long time span some plants spent in the vegetative stage was reflected by the low transition probabilities of the plants in this life cycle stage. Clearly, delaying reproduction to remain in the vegetative stage increased the likelihood of mortality. However, delayed reproduction may have been necessary to give plants time to attain a sufficient allocation of resources to produce seed (Silvertown 1987). Fecundity was highly variable between plants (i.e. 95 to 19 596 seeds per plant) and was correlated to plant size rather than life span. *Salsola australis* plants continued juvenile and adult growth once reproduction had commenced. This may have allowed plants to enter reproduction once minimum resources required for reproduction were available (Silvertown 1987) and then take advantage of any subsequent resource availability to increase plant size and total fecundity.

Plants became mobile if they died in the adult vegetative growth stage (regardless of seed production), and degraded without becoming mobile if they died in the seedling or juvenile vegetative growth stage. This indicates that an abscission layer develops at the base of the stem during or after the transition to adult vegetative growth, allowing the above ground plant biomass to be released from the roots following senescence. In the crop plot, plants only became mobile in summer/autumn because the cropping process

prevented plant growth over the winter months. In the pasture and fallow plots, plants also became mobile in winter and spring, generally in the months of lowest rainfall. When plants did become mobile in months of medium to high rainfall (i.e. June and September), there was equally high rainfall in the preceding month. It is possible that the release of plants was delayed by high soil moisture levels. Senescence and abscission of other plant organs is related to water stress resulting from either a surplus or a deficient of available water, although the relationship between water stress and abscission is not fully understood (Brown 1997b; Roberts et al. 2002; Taylor and Whitelaw 2001). Accumulations of senesced *S. australis* plants against fences or other fixed objects are a common feature of the Western Australian wheat-belt in autumn, but this is clearly because the predominant land use is winter cropping.

Effective weed control options have not been identified for *S. australis* in southwestern Australian agricultural systems, and further research is required to identify and assess possible management options. Identification of profitable areas of research can be achieved through weed control models. The data in this chapter, combined with the assessment of the soil seed bank and seed dispersal data presented in Chapters 5 and 6, give an outline of the population ecology of *S. australis* in the Lake Grace region. Further research in Chapter 8 compiles this data into a model of the life cycle of *S. australis*, to simulate the effect of various control techniques on the population growth rate within agricultural systems in southwest Australia. This work was not replicated over time as it was necessary to cease observations after November 2005. A severe flood prevented access to the region for several weeks and disrupted the trial sites. While the trial was replicated over location, there are likely to be differences between the populations at more widely dispersed regions, subjected to different agricultural practices. Further research is required to determine how much the population ecology of *S. australis* varies throughout Western Australia.

# Chapter 8

# Assessment of management options for *Salsola australis* R.Br. in the southwest Australian agricultural system by transition matrix modelling


**Abstract**

A model of the life cycle of *Salsola australis* was constructed, based on population ecology data collected from the district of Lake Grace, Western Australia. The model was used to assess control strategies to use against this summer annual weed within the broad acre grain cropping system of the Western Australian wheat-belt. The population growth rate of *S. australis* in the absence of weed control strategies was 1.49. Elasticity analysis indicated that population growth rate was mainly dependant on the production of adult plants in year two by adult plants present in year one, and was virtually unaffected by the dormant seed bank. The model determined that physical weed control, through removal or burning of all senesced plants, would reduce population growth rate to 0.55 and result in a 100% chance of the population becoming extinct in 25 years. Residual herbicide control resulting in 57% reduction in plant survival would reduce population growth rate to 0.99 and the population would have a 22.8% chance of becoming extinct in 25 years. Biological control with the rust fungus *Uromyces salsolae* would reduce population growth rate to 0.94. However, this was based on the assumption that this fungus would be equally pathogenic on *S. australis* populations in field conditions as it was in controlled conditions. If the fungus is less effective in field conditions, it would not stop population expansion. Weed control strategies were not effective if seed migrated to the population in question from neighbouring populations of *S. australis* through broad scale seed dispersal. This research concluded that effective weed management depended on preventing seed dispersal from neighbouring *S. australis* populations rather than attempting to reduce the dormant seed bank. Further research is required to validate the assumptions in the model and determine the effectiveness and practicality of the proposed management strategies in the field.


## Introduction

In considering the range of factors influencing plant growth, there are many advantages to building a model to capture the complexity. Constructing a model necessitates the

assimilation and integration of data for a given species, which clarifies assumptions and indicates where knowledge is deficient (Akcakaya et al. 1997; Possingham et al. 1993). Models allow us to identify the parameters of population dynamics that have the most effect on a given species and so allows us to explore the results of management options to preserve or control the species (Akcakaya et al. 1997; Caswell 2001). Models have been used to determine how best to manipulate population dynamics of both annual and perennial weed species, and estimate the long-term effects of weed management strategies (Holst et al. 2007; Neve et al. 2003; Odom et al. 2003; Rees and Paynter 1997).

Two time scales should be considered when constructing a model for an annual weed. All demographic processes in the life cycle of the species occur within a year (rather than between years as for a perennial species), but population growth rate needs to be considered between years. Models of annual species have ignored this issue by focussing on a single time scale. Some models have focused on within year dynamics (Cousens and Mortimer 1995; Silvertown and Lovett-Doust 1993). Other models have collapsed the annual life cycle at some point in the year (i.e. the probability that a seed will produce more seeds) to examine between year dynamics (Watkinson 1980; Watkinson et al. 1989). Yet another approach is to focus on the dormant seed bank of an annual plant, which effectively transforms an annual life cycle into a perennial life cycle (i.e. a seed exists for several years before producing more seed) (Jansen et al. 1996; Kalisz and McPeek 1992). However, models can take into account both time scales. Griffith and Forseth (2005) produced a model of an annual that focused on the perennial component, the dormant seed bank. However, they analysed the effect of within year demographic processes as lower order parameters and the between year processes as higher order parameters in a matrix model. Caswell (2001) presented a transition matrix model that examined the effect of within year demographic processes on between year population growth. It is beneficial to consider the within and between year demographic processes in the life cycle of an annual. Within year processes determine the population growth rate, but population growth rate must be assessed over a long time span to assess population dynamics and the effect of management options.

*Salsola australis* is a common weed in southwest Australia. The agricultural system in the wheat-belt of Western Australia consists of annual broad scale grain crops and ley

pasture systems, often in rotation. *Salsola australis* is a summer annual weed in both the cropping and pasture systems (Mussell and Stewart 2004). The population ecology of *S. australis* within this agricultural system, including characteristics of the life cycle, seed biology and seed dispersal, has been the subject of recent study (Chapter 5, 6 and 7). While there are management strategies currently practiced in Australia to control *S. australis*, there is no research available on the short or long term effects of these weed control strategies. However, there is a significant amount of research internationally on the management of similar species of the *Salsola* genus in broad scale annual cropping systems (Anderson et al. 1998; Blackshaw et al. 1994; Blackshaw and Lindwall 1995; Harbour et al. 2003; Hasan et al. 2001; Smith 2005; Young and Gealy 1986; Young and Whitesides 1987). A deterministic matrix population model was constructed using the available population ecology data, and used to assess the effectiveness of various physical, chemical and biological control techniques on the population growth rate and invasion speed of *S. australis*.

**Model formation**

*Model selection*

The population dynamics of *S. australis* were investigated using a population matrix model based on the model developed by Kalisz and McPeek (1992). This model takes into account the factors affecting survival within each year as well as the dormant seed bank. The data used to construct this model was predominately obtained from trials conducted in the district of Lake Grace, Western Australia, from 2004 to 2006 (Chapter 5, 6 and 7). The *S. australis* populations in question were growing as weeds in a broad scale annual cropping system. The model assumes an annual cropping event, as that is the predominant land use in the wheat-belt of Western Australia.

*Within year life cycle dynamics*

*Salsola australis* plants progress through the life cycle stages of seeds, seedlings and plants in the vegetative or reproductive stages, before senescence (Table 8.1, Figure 8.1, Chapter 7). The senesced plants break free of their root systems to become mobile, and the seeds either remain attached to the mobile plants (retained seeds) or are shed from the plants (easily shed seeds). In the agricultural system, the mobile plants are routinely crushed and incorporated into the soil seed bank before or during the process of sowing

a crop. So both easily shed and retained seeds can germinate in the year following seed production, or enter the dormant seed bank.

The life span of *S. australis* plants varies from 2 to over 12 months. All life cycle stages are present throughout the year. However, within the grain cropping system, it is possible to collapse the annual life cycle. Seedlings establish from August (i.e. in winter, within the crop) to May of the following year (i.e. autumn, prior to sowing the next crop). So plants grow from winter to the following autumn and by this point most of the population is at or has previously reached senescence. When the crop is sown in April or May, all *S. australis* plants are killed by cultivation and herbicides. Herbicides applied at the time of sowing, and in-crop herbicides applied later in the season, prevent further cohorts from establishing until July-August. The transition probabilities (i.e. the probabilities of surviving from one stage to the next stage) shown in Table 8.1 are averages taken from all cohorts growing throughout a year from July-August to April-May.

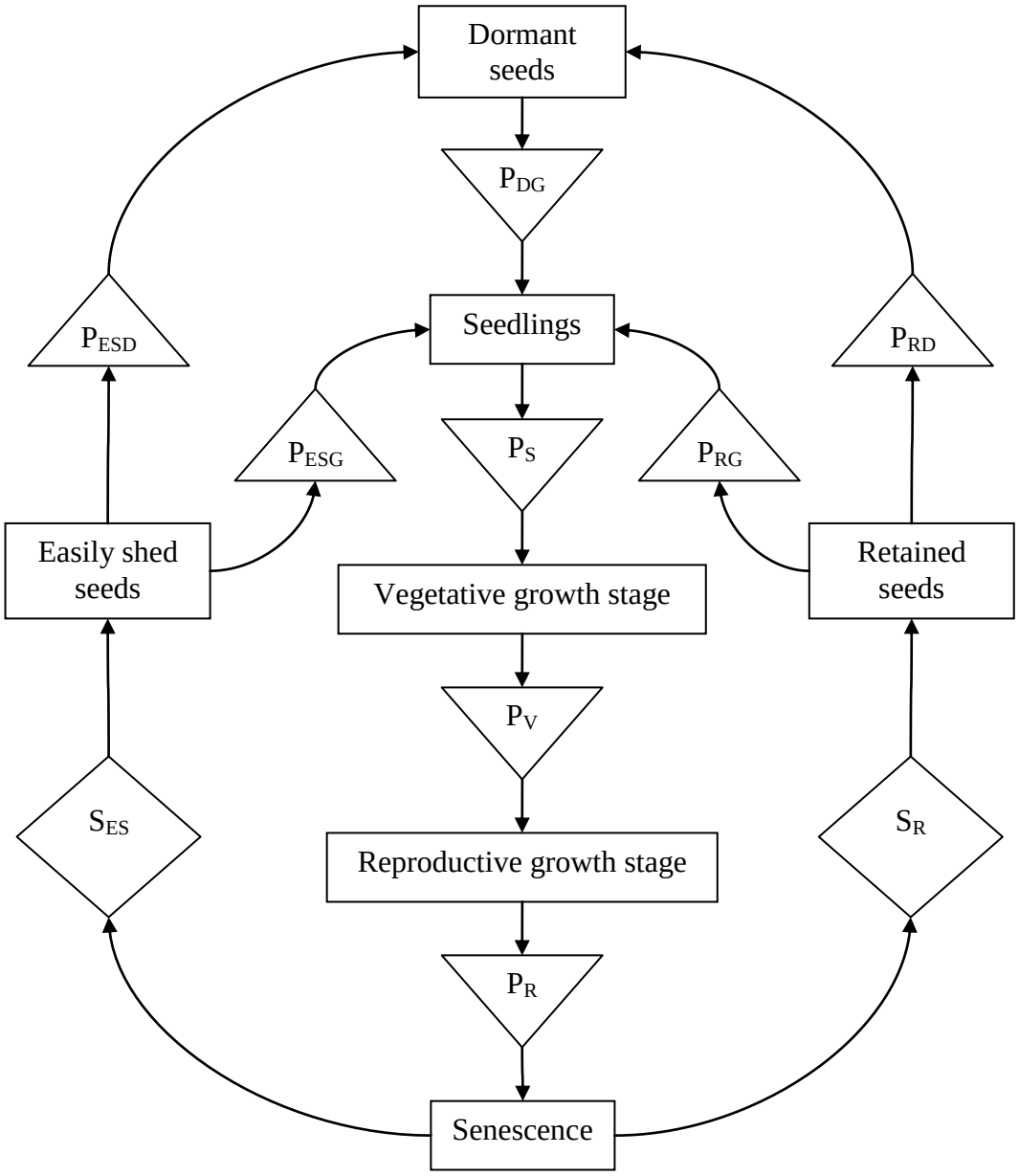


Figure 8.1: Diagrammatic life table of *S. australis*. The rectangles represent stages of the life-cycle, including seedlings, plants in the vegetative, reproductive or senesced stage of the life cycle, seeds retained on the plant, seeds that are easily shed from the plant and dormant seeds. The triangles represent the survival probabilities of each life cycle stage, including seedlings to vegetative plants ($P_S$), vegetative to reproductive plants ($P_V$), reproductive to senesced plants ($P_R$), probability of easily shed or retained seeds germinating in the year following seed production ($P_{ESG}$ and $P_{RG}$), probability of easily shed or retained seeds becoming dormant ($P_{ESD}$ and $P_{RD}$) and the probability of dormant seeds germinating ($P_{DG}$). The diamonds represent fecundity, i.e. total viable easily shed or retained seed produced per plant ($S_{ES}$ and $S_R$).

Table 8.1: Mean and standard deviation of each parameter used to describe the life cycle of *S. australis* defined in Figure 8.1.

| Parameter | Definition | Mean | SD |
|---|---|---|---|
| $S_{ES}$ | Total number of viable, easily shed seeds per plant | 72.12 | 69.09 |
| $S_R$ | Total number of viable, retained seeds per plant | 100.91 | 96.65 |
| $P_{ESG}$ | Probability of easily shed seed germinating in the year following seed production | 0.1464 | 0.0446 |
| $P_{ESD}$ | Probability of easily shed seed staying dormant in the year following seed production | 0.8557 | 0.0446 |
| $P_{RG}$ | Probability of retained seed germinating in the year following seed production | 0.0261 | 0.013 |
| $P_{RD}$ | Probability of retained seed staying dormant in the year following seed production | 0.9739 | 0.013 |
| $P_{DG}$ | Probability of dormant seeds germinating | 0.0185 | 0.0036 |
| $P_S$ | Survival probability of seedlings | 0.9 | 0.1287 |
| $P_V$ | Survival probability of vegetative plants | 0.28 | 0.0108 |
| $P_R$ | Survival probability of reproductive plants | 0.39 | 0.0542 |

*Between year life cycle dynamics*

Modelling of within year population dynamics is difficult to manage accurately, due to the overlapping generations of plants. However, in autumn when the crop is sown, all plants have finished producing seed or are killed by the cropping enterprise. At this stage of the year the life cycle can be collapsed on an annual time span (Figure 8.2). Adult plants in autumn produce seed that either germinates to produce adult plants by the following autumn (one year old adults, Adult 1), or remains dormant and so is a one year old seed in the following autumn (Seed 1). In the following year, this dormant seed may germinate to produce an adult plant that is two years old, i.e. the plant is one year old and the dormant seed that produced the plant was one year old (Adult 2). Both the one year old (Adult 1) and the two year old (Adult 2) plants can produce further one year old (Adult 1) plants or one year old dormant seed (Seed 1) in the next year. It was assumed that the seed bank lasted one year. While there is no data available on this, there is evidence to suggest that the seeds of other, morphologically similar species of the *Salsola* genus only last for a year or two in field conditions, although they can

survive for several years in laboratory conditions (Evans and Young 1980; Fowler et al. 1988).

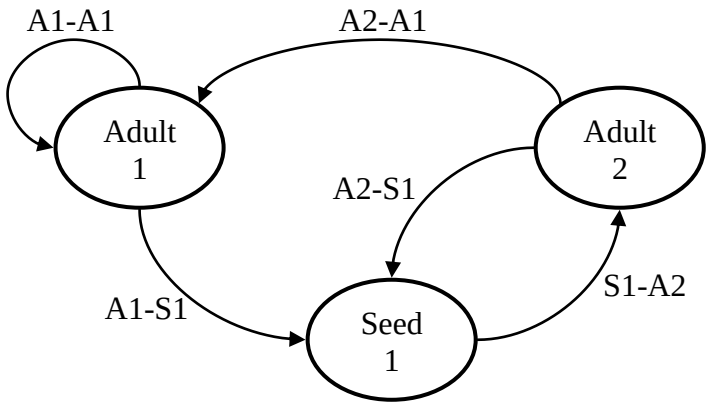


Figure 8.2: Life cycle graph for *S. australis,* assuming a 1 year seed bank. Each arrow represents an autumn to autumn transition. Adult 1 indicates plants that are one year old, produced by (Adult 1 or Adult 2) plants in the previous year. Seed 1 indicates dormant seed that has been in the seed bank for one year, produced by (Adult 1 or Adult 2) plants in the previous year. Adult 2 indicates plants that are two years old, as the plants are one year old and the dormant seed (Seed 1) that the plants generated from was one year old. The labels associated with each arrow are abbreviations for the adult or seed stages at the start and end of each transition. The values of the arrow labels are the products of survival probabilities, or survival probabilities and fecundity, identified in Figure 8.1 and defined in Equation 8.1, 8.2 and 8.3.

It was assumed that the probability of an adult plant producing more adult plants would be consistent between Adult 1 and Adult 2 plants (A1-A1 = A2-A1, Figure 8.2). It was also assumed that Adult 1 and 2 plants had an equal probability of producing dormant seed (A1-S1 = A2-S1). Given that these values were equal, they could have been combined in the model. They are kept separate to distinguish the effect of the dormant seed bank on population growth rate. These transition probabilities are calculated from the life cycle values defined in Figure 8.1, as shown in Equations 8.1, 8.2 and 8.3.

Equation 8.1: $A1\text{-}A1 = A2\text{-}A1 = ((S_{ES} \times P_{ESG}) + (S_R \times P_{RG})) \times Ps \times Pv \times P_R$

Equation 8.2: $A1\text{-}S1 = A2\text{-}S1 = (S_{ES} \times P_{ESD}) + (S_R \times P_{RD})$

Equation 8.3: $S1\text{-}A2 = P_{DG} \times Ps \times Pv \times P_R$

The transition parameters for each stage in the life cycle of an *S. australis* population with a one year seed bank (defined in Figure 8.2 and Equation 8.1, 8.2 and 8.3) were incorporated into the transition matrix A.

| A | Seed 1 | Adult 1 | Adult 2 |
|---|---|---|---|
| Seed 1 | 0 | A1-S1 | A2-S1 |
| Adult 1 | 0 | A1-A1 | A2-A1 |
| Adult 2 | S1-A2 | 0 | 0 |

The transition matrix was used to construct the transition matrix model shown in Equation 8.4, which can be used to project the population size. The column vector $N_{t+1}$ describes the number of Seed 1, Adult 1 and Adult 2 individuals at time t + 1. It is a function of the transition matrix A and the vector $N_t$ (number of individuals at time t).

Equation 8.4: $N_{t+1} = A \times N_t$

Equation 8.4 is written in full as:

$$\begin{pmatrix} \text{Seeds 1} \\ \text{Adults 1} \\ \text{Adults 2} \end{pmatrix}_{t+1} = \begin{pmatrix} 0 & \text{A1-S1} & \text{A2-S1} \\ 0 & \text{A1-A1} & \text{A2-A1} \\ \text{S1-A2} & 0 & 0 \end{pmatrix} \times \begin{pmatrix} \text{Seeds 1} \\ \text{Adults 1} \\ \text{Adults 2} \end{pmatrix}_{t}$$

**Population projections**

This model indicates λ (population growth rate) was 1.49 (i.e. the population was increasing by approximately half every year). The stable age distribution was dominated by dormant seeds (i.e. 0.99 Seed 1 individuals vs. 0.008 Adult 1 and 0.0012 Adult 2 individuals). However, the reproductive values (i.e. contribution of Seed 1, Adult 1 and Adult 2 to future generations relative to that of Seed 1 individuals) were much higher for the adults (94.9 for both Adult 1 and Adult 2 individuals compared to 0.11 for Seed 1 individuals). Population growth over 10 years under two simulations is shown in Figure 8.3. The first simulation assumes that a single Adult 1 plant is found in the region at the beginning of the time period. The second assumes that 98 Seeds 1 dormant seeds were initially found in the region. If a single mobile plant entered a field, and was crushed through the cultivation process that occurs at sowing, 98 attached, viable seeds

would be incorporated into the dormant soil seed bank. For both simulations the population expands exponentially after initial fluctuations in the first two years.

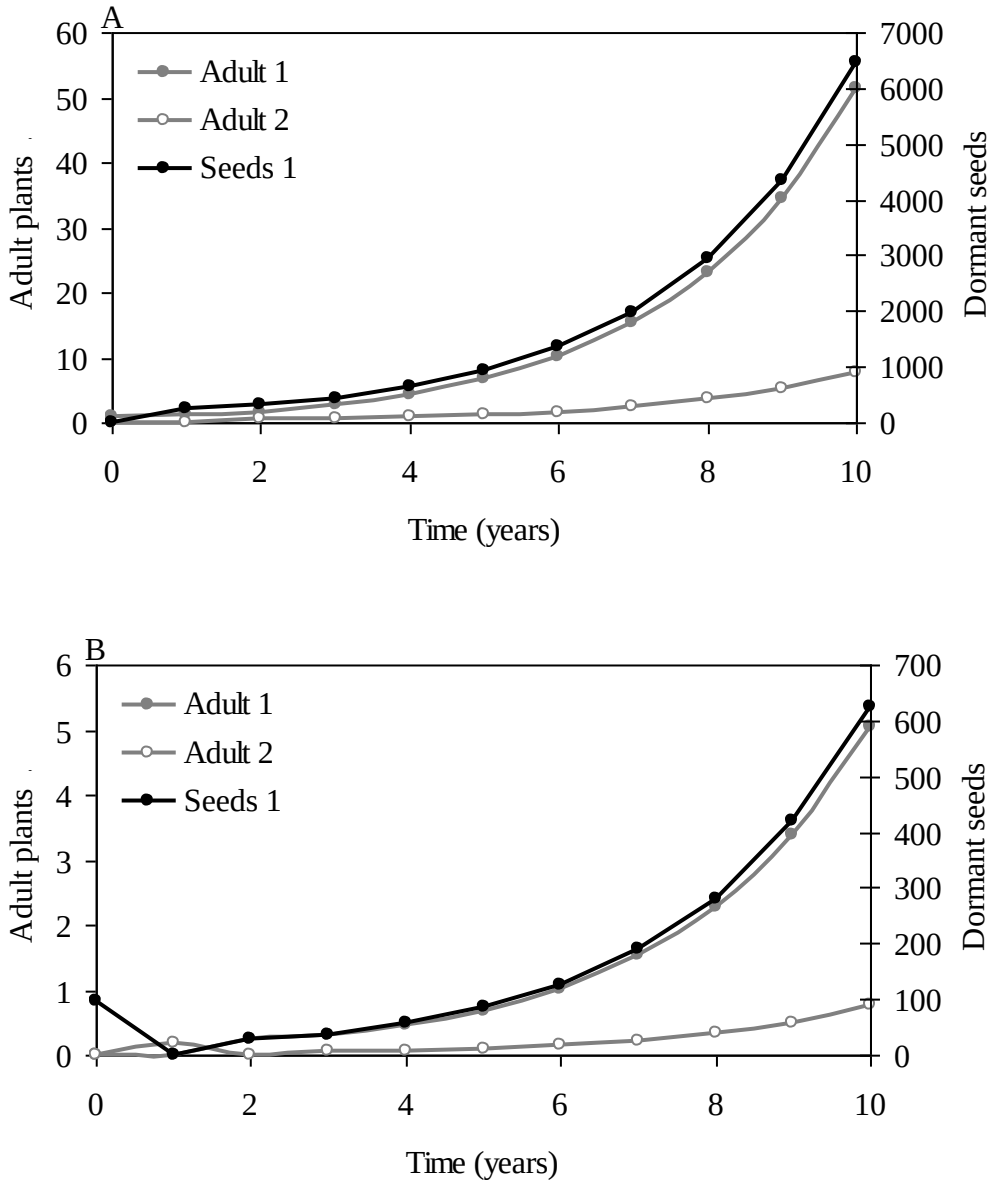


Figure 8.3: Population growth model for *S. australis* over 10 years, using Equation 8.4. Population growth is simulated by assuming a single Adult 1 plant (A) or 98 Seeds 1 dormant seeds (B) are present at time zero years. The total number of Adult 1 and Adult 2 plants in the population is plotted on the left axis and the total number of Seeds 1 dormant seeds is plotted on right axis.

*Sensitivity analysis*

The sensitivity and elasticity of λ with respect to changes in the demographic parameters indicate the impact of the parameters on λ. Sensitivity indicates the additive or absolute change in λ resulting from a change to a parameter, i.e. slope of λ as a function of the parameter. Elasticity indicates the relative change in λ resulting from a change to the parameter, i.e. slope of log λ as a function of the log of the parameter. The sensitivity and elasticity of both transition probabilities within the matrix model (A), and the lower level parameters used to calculate the matrix transition probabilities (in Equation 8.1, 8.2 and 8.3, Figure 8.1) were calculated according to the methods in Caswell (2001).

The sensitivity of λ to the matrix transition probabilities was greatest in relation to the fate of Seed 1 dormant seeds (Figure 8.4). However, the elasticity of λ was greatest in the transition of Adult 1 plants to Adult 1 plants. Seed 1 and Adult 2 individuals had very little effect on the population. This indicates that all control measures should focus on disruption of the within year population dynamics that affect the transition A1-A1 (i.e. seed production, germination of seed and transition probabilities between life cycle stages). This is confirmed by the elasticity of λ to lower level parameters shown in Table 8.2. However, the elasticity of λ to the number of retained seeds produced or the probability of the retained seeds germinating was very low compared that of the easily shed seeds.

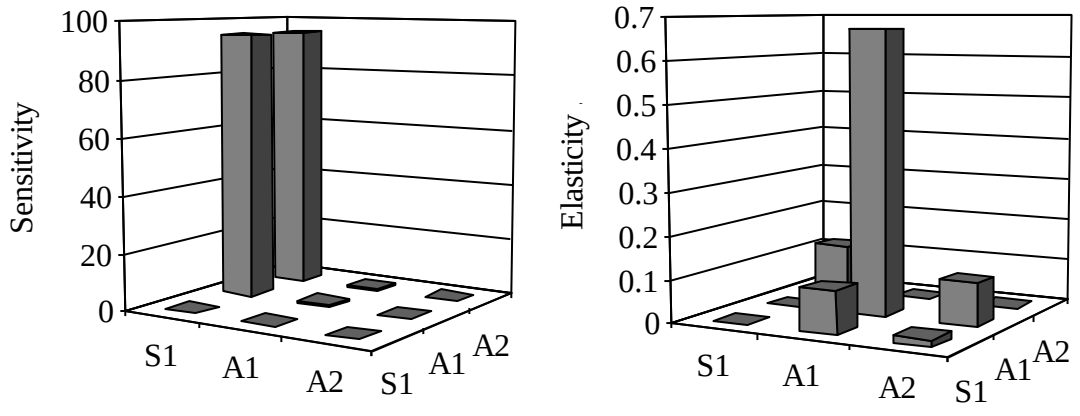


Figure 8.4: Sensitivity matrix (left) and elasticity matrix (right) of λ to individual matrix transition probabilities. The columns in the graphs correspond to the transition probabilities shown in transition matrix A.

Table 8.2: Sensitivity and elasticity of λ to individual lower level parameters (Figure 8.1) used to calculate the matrix transition probabilities.

| Parameter | Sensitivity | Elasticity |
|---|---|---|
| $S_{ES}$ | 0.014 | 0.660 |
| $S_R$ | 0.003 | 0.224 |
| $P_{ESG}$ | 6.269 | 0.615 |
| $P_{RG}$ | 8.772 | 0.153 |
| $P_{ESD}$ | 0.078 | 0.044 |
| $P_{RD}$ | 0.109 | 0.071 |
| $P_S$ | 1.466 | 0.884 |
| $P_V$ | 4.711 | 0.884 |
| $P_R$ | 3.382 | 0.884 |
| $P_{DG}$ | 9.327 | 0.115 |

**Simulation of management scenarios**

*Environmental and demographic stochasticity*

To simulate population growth under various management scenarios, stochasticity was incorporated into the model. To simulate environmental stochasticity, seed production and probabilities (for survival, seed dormancy and seed germination) were considered as random variables with normal distributions (Hiraldo et al. 1996). For each simulation, the value of the parameters was calculated using Equation 8.7, where p is the parameter value for a given year in a given simulation, x is the mean value of the parameter, s is the standard deviation of the parameter (which are given in Table 8.1) and d is the normal standard deviation. This value varied randomly for each simulation. The model assumes a perfect correlation between survival probabilities and seed production, i.e. if the probability of a plant surviving is lower than average then seed production will also be lower than average (to simulate a "bad" year).

Equation 8.7: $p = x + (s \times d)$

Demographic stochasticity was simulated by sampling Poisson distributions to determine the number of seeds produced per plant and binomial distributions to determine the number of plants that survive and number of seeds that become dormant or germinate each year (Akcakaya et al. 1997). Each model was run for 1000

replications to determine average population growth under various management scenarios, over a 25 year period.

*The agricultural system*

Seed dispersal in the agricultural system is limited. Cropping fields in the Western Australian wheat-belt are usually fenced, with a wire mesh fence around 1 m high, to contain grazing livestock (as cropping is rotated with pasture systems). Most mobile *S. australis* plants are contained in the field in which they originate, but approximately 25% of the plants are blown over the fence to migrate into the neighbouring fields downwind of the population (Chapter 6). In the population projection above, the number of retained seeds is not varied. It is assumed that the 25% of plants (with attached seeds) moving to the field downwind of the population in question is compensated for by the 25% of plants entering from the field upwind of the population.

Possible control techniques used against *S. australis* in Australia include physical, herbicide and biological control techniques. Weed control was simulated by altering the lower level parameters in Equations 8.1, 8.2 and 8.3 as specified below in the descriptions of each control technique, which altered the parameters in the transition matrix model (Equation 8.4). However, for each simulation of a control technique, the number of retained seeds was varied. It was still assumed that 25% of the plants migrate to the field downwind of the population each year. However, the field upwind of the population is assumed to contain from zero to 100 plants. From Table 8.1, each plant carries 100.91 viable, retained seeds. Assuming 25% of a population of 100 plants escape the field, then 2523 retained seeds are carried into the field in question. A neighbouring population of 100 plants would be a very small, sparse population of *S. australis* in an agricultural field, or a normal population observed on the roadside next to the field. In the absence of control measures, assuming that 25% of retained seeds leave the field and 2523 seeds from a neighbouring population of 100 plants enter the field, $\lambda$ is increased to 8.21.

*Physical control*

In an annual cropping system physical control could be achieved through removal or burning of senesced plants in autumn before the plants become mobile, instead of using the cultivation that occurs at time of sowing to crush all senesced and mobile plants and

incorporate them into the soil. This must be done before plants become mobile, as the mobile plants present a fire hazard. Physical control was simulated by assuming that easily shed seeds were controlled, except for the 24% that are released before senescence, and retained seeds were controlled, except for the 2523 retained seeds from the neighbouring population of 100 plants. Under this management scenario, λ was 7.53. In fact, if the neighbouring population contained more than 4 plants (i.e. 101 retained seeds entering the field), λ was greater than one under this weed control method. However, if it was assumed that this control technique was applied to the whole region (i.e. no plants can migrate from the neighbouring population) or assumed that there were no neighbouring plants, λ was reduced to 0.55 and so the population was declining towards extinction rather than expanding (as λ was less than 1, not greater than 1). The population trajectory summary over 25 years under this management regime is shown in Figure 8.5 and the probability of the population becoming extinct within this period was 100%.

*Herbicide control*

If it was assumed that there were no neighbouring plants, herbicides reducing seed production by 40% or more resulted in a λ of less than 1 (Figure 8.5), and a 22.8% chance of the population becoming extinct over 25 years. Use of herbicides to reduce seed production made virtually no difference if retained seeds from neighbouring fields continued to enter the system. When seed production of the population was reduced to zero and migrating seeds from a neighbouring population of 100 plants were the only additions to the seed bank, λ was 7.10. In fact, with 100% seed set control, the neighbouring population needed to consist of nine plants for λ to be 0.99 (i.e. population remaining at a stable size). The neighbouring population needed to be less than nine plants to ensure the population was declining rather than remaining stable. However, herbicides applied at several times of the year, or residual herbicides applied after crop harvest in November could reduce the probability of plant survival. When the survival probabilities of plants in the seedling, vegetative and reproductive stages were reduced by 57% or more, λ was less than 1, in spite of retained seed from a population of 100 plants migrating into the region.

*Biological control*

Biological control is not used in Australia against *S. australis*. However, a rust fungus (*Uromyces salsolae*) is a potential biological control agent, and is found in isolated locations in the eastern States and Western Australia (Hasan et al. 2001). Preliminary investigations into the effectiveness of this biological control agent in controlled conditions in the USA indicated that for *S. kali* (possibly *S. australis*) plants, this rust fungus can increase plant mortality by 54.5% and prevent seed production in 100% of infected plants. Biological control was simulated by assuming that half of all *S. australis* plants in the field became infected with the fungus. So survival to reproductive maturity was reduced by 27.25% and seed production from the remaining plants was reduced by 25%. This resulted in a $\lambda$ of 0.94, and a 36.6% probability that the population would become extinct over 25 years. It was assumed that this control method was applied to all *S. australis* populations in the region (i.e. the population in question and any neighbouring plants), since a rust fungus would not be limited to a single field or population (Figure 8.5).

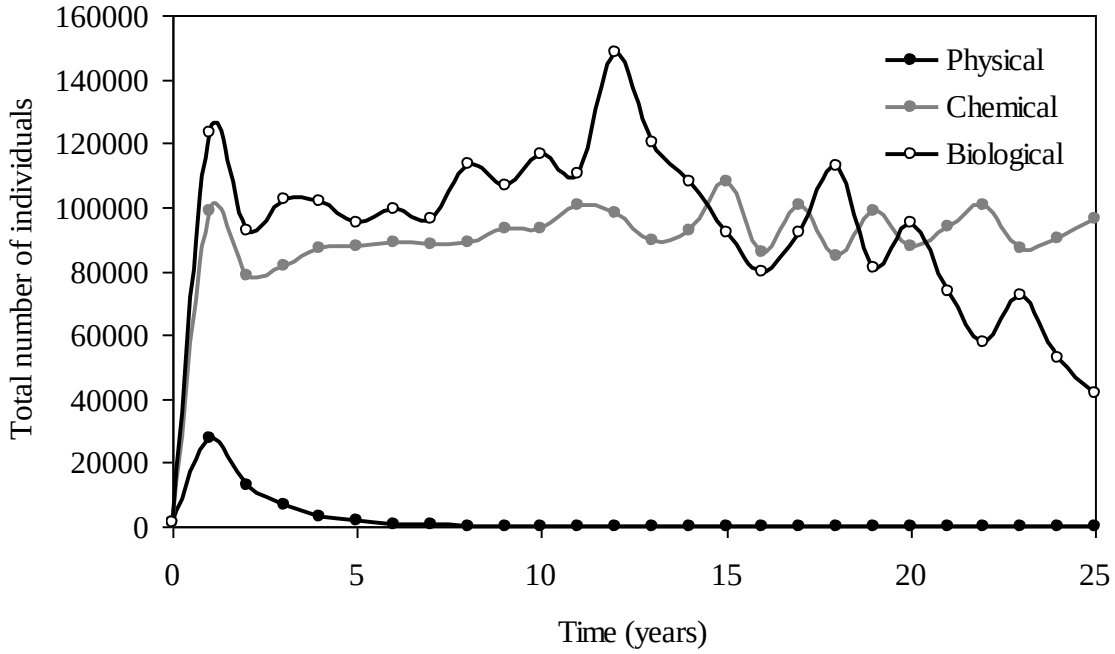


Figure 8.5: Total number of combined Adult 1, Adult 2 and Seed 1 individuals in a population of *S. australis* with an initial abundance of 1000 Adult 1 plants over 25 years, exposed to physical, chemical or biological control measures. Physical control assumes that all seed is destroyed (through burning senesced plants) except for the 24% of seed that comes loose before plants are fully senesced. Chemical control assumes that herbicides reduce seed production by 40%. Biological control assumes that the rust fungus reduces plant survival by 27.25% and remaining seed production by 25%. All simulations assume that control measures were applied to the population in question and to neighbouring populations.

**Discussion**

The population growth rate indicated that the *S. australis* population at Lake Grace was expanding exponentially (due to the lack of density dependant regulators) rather than declining. This is surprising given the very low rate of seed viability and low probability of germination. The reason for the extremely low seed viability observed in this population of *S. australis* in the region of Lake Grace has not been explained. It is significantly lower than that of most other populations of *S. australis* in Western Australia (Chapter 3, 5) and other, morphologically similar species of the *Salsola* genus (Evans and Young 1972; 1980; Fowler et al. 1988). However, the low seed viability was not causing the population to approach extinction. The growth rate was influenced to the greatest extent by the transition of Adult 1 to Adult 1 plants, i.e. adult plants in year two

produced from adult plants in year one (as opposed to adult plants produced from the dormant seed bank). This was not surprising given the very low probability of germination of dormant seed. The production and germination of easily shed seed, and the survival of plants predominately influenced population growth rate. So control techniques that aim to reduce these values, without focussing on reducing the dormant seed bank, are likely to be the most successful.

The management of other annual, agricultural weeds in the Western Australian cropping system has been dependent on reducing total seed production to reduce the seed bank (Monjardino et al. 2003; Pannell et al. 2004), but *S. australis* management should not focus on the dormant seed bank. A short lived soil seed bank has been found in other species of the *Salsola* genus (Evans et al. 1982; Evans and Young 1980; Sankary and Barbour 1972; Young 1991; Young and Evans 1979). Seed banks are useful in ensuring populations against years of poor seed production, but a seed bank results in increased seed mortality and delayed reproduction, which reduces the potential population growth rate (assuming that all seedlings have an equal chance of survival) (Fenner and Thompson 2005). While short-lived seed banks are a common feature of weed species within the *Salsola* genus, these species engage in broad scale seed dispersal (Mallory-Smith et al. 1993; Stallings et al. 1995; Warren 2001) (Chapter 6). It has previously been found that broad scale seed dispersal is a trait that develops at the expense of long term seed dormancy (Rees 1993). Seed dispersal ensures that seeds germinate in different locations, while seed dormancy ensures that seeds germinate in different years. Both strategies ensure that the seedlings face heterogeneous environments, and so the likelihood of all seedlings facing an unsuitable environment is reduced (Fenner and Thompson 2005; Rees 1993). This was confirmed by the model. The dormant seed had very little effect on the population growth rate, but retained seed entering the field from a neighbouring *S. australis* population via broad scale seed dispersal had a large impact on the population.

Seed dispersal significantly affected population growth rate and the success of weed control strategies. The maximum neighbouring population of 100 plants incorporated into this model was a very small population of *S. australis*. In an agricultural field, 100 plants could be found over an area of less than 25 $m^2$. However, the migrating seed from this population was sufficient to increase the population growth rate from 1.49 to 8.21

and to ensure that any control measure applied to a single field was ineffective. In fact, in the absence of weed control measures, the yearly addition of a single plant from a neighbouring population into a field previously free of *S. australis* was sufficient to ensure that the population established and expanded. In established populations, fewer than ten plants in the neighbouring field were sufficient to allow the population to expand even if weed control practices were used. The variation in population growth rate due to the addition of seed from other populations highlights the importance of applying control measures to an entire farm (including roadsides), or to the entire district, rather than to a single field.

Physical weed control through burning crop residue in an attempt to burn weed seeds lying on the soil surface is a control measure currently practiced in the Western Australian wheat-belt (Cheam and Code 1995; Gill and Holmes 1997). No data exists on the feasibility of burning *S. australis* plants at the end of the year. However, it is likely to be highly effective given that the seeds are only protected by a thin, dry, highly flammable fruiting perianth (Chapter 5). Burning *S. australis* plants would be most feasible after plants reached senescence but before they became mobile. At this stage, 24% of seeds have already shed, but most seed is still attached to the plant. This would require burning to occur in early autumn, which would present a fire hazard. However, burning plants in late autumn would also present a fire hazard as the plants are mobile and may cause the fire to spread. Some farmers currently rake the mobile plants into compacted piles to be burnt or to remove them before the crop is sown (Graham Mussel, Department of Agricultural and Food Western Australia, pers. comm.). However, this may not be an effective weed control technique. The agitation of the raking process would likely cause a considerable proportion of seeds to shed from the mobile plants and thereby escape the burning process. The model indicated that burning all plants before they became mobile was a highly effective way to reduce population growth rate, as long as seed from neighbouring populations could not disperse into the region once the burning process was complete. Other methods of physical control such as cultivation and grazing were not examined here. Cultivation was highly effective in removing *S. australis* plants (Chapter 7), but a zero tillage cultivation system has been adopted in southwest Australia to conserve soil structure and soil moisture (Turner 2004; Turner and Asseng 2005). The introduction of cultivation over the summer fallow to control *S. australis* would not be economically viable. Grazing only affected young plants, as

stock will not eat the mature plants at the reproductive stage, and so grazing did not affect total seed production (Chapter 7).

Herbicide control of *S. australis* is challenging. Firstly, continuous cohorts of seedlings appear throughout the year and it is difficult to target all plants (Chapter 7). Secondly, herbicides are not very effective when applied to plants beyond the seedling stage, with commonly recommended herbicides controlling 50-90% of plants (Mussell and Stewart 2004). This is possibly because most plants grow as summer annuals in arid, stressful conditions, and exposure to environmental stress makes many weed species herbicide tolerant (Harvey and Crothers 1988; Lanini and Radosevich 1982). The model indicates that herbicide control was relatively easy if herbicides were applied to all populations in a region, since a reduction in seed set of 40% or above was required to reduce the population growth rate to less than one and cause the population to approach extinction. This can be achieved with herbicides such as glyphosate, 2,4-D amine, paraquat and diquat (Mussell and Stewart 2004; Young and Thorne 2004; Young and Whitesides 1987). However, as for physical control, reduction in seed set had virtually no effect if additional seed migrates into the area. In this case, survival probability of plants needed to be reduced by 57% or more to cause the population growth rate to fall below one. Given the number of cohorts of seedlings appearing throughout the year, reducing these survival probabilities could most easily be achieved through use of residual herbicides (from the sulfonylurea chemical group). These herbicides are highly effective against species of the *Salsola* genus, including *S. australis* (Mussell and Stewart 2004; Young and Gealy 1986). However, residual herbicides cannot be used in all systems due to crop compatibility issues. On alkaline soils where *S. australis* is abundant, residues of residual herbicides is of particular concern (Hollaway et al. 2006; Mitchell and Wilcox 1988).

The biological control agent *Uromyces salsolae* has already been found on *Salsola* populations in Australia as well as many other continents (Hasan et al. 2001). However, this fungus has only been evaluated in controlled glasshouse conditions, and to effectively control *S. australis* it would need to infect at least half of the population and act as effectively in field conditions as it does in controlled conditions. There are other biological control agents in use or under evaluation for use against various species of the *Salsola* genus (Bruckart et al. 2004; Smith 2005; Sobhian et al. 2003). However, it is

unlikely that biological control agents not already found in Australia would have any chance of being released against *S. australis*, given the low economic impact of weeds of the summer fallow period compared to weeds that grow in the winter cropping season. Further, the origin of the *Salsola* genus in Australia remains in doubt (Bean 2007). The possibility that these plants might be native would prevent the introduction and use of exotic pathogens as biological control agents.

This research concludes that physical, herbicide or biological weed control techniques may be successful in reducing populations of *S. australis* growing in agricultural systems. However, the success of weed management strategies clearly depends on the extent of seed dispersal between populations. Attempting to reduce population growth through weed control while neighbouring populations are not controlled was futile. Further, while this model focuses on the cropping system, cohort survival and seed production was greater in a pasture system (Chapter 7). Therefore, seed production and population growth rate may be altered in a cropping and pasture rotation, compared to the cropping system considered here. Clearly, further research on *S. australis* population dynamics in varying agricultural systems, and the effectiveness and practicality of weed management techniques in field conditions is required to develop successful control strategies.

# Chapter 9
# Discussion

Species of the genus *Salsola* are widespread and successful weeds of native vegetation and ruderal habitats throughout Australia and overseas (Beadle 1981; Eldridge et al. 2006; Mitchell and Wilcox 1988; Rilke 1999a; Westbrooke and Florentine 2005). However, within Australia, very little information was available on this genus. An initial aim of this thesis is to gain an understanding of the genetic diversity, biology and ecology of the genus *Salsola* in the agricultural region of southwest Australia. A secondary aim is to use this information in identifying potential management strategies for this weed in these systems. Within this final chapter, the main findings of this thesis are summarised and the implications of these conclusions are discussed, followed by suggestions for further work to improve understanding and management of the genus *Salsola* in Australia.

**Main conclusions**

The review of the literature on *Salsola* indicated how widespread the species *S. tragus sensu lato* is throughout Australia, and highlighted the taxonomic confusion evident for this species in Australia and worldwide. The review identified the paucity of information on the biology and ecology of Australian populations of this species, especially as it occurs as a weed in agricultural systems. Further, the impact of this weed in agricultural regions and suitable management options were poorly understood in Australia. The literature review resulted in research objectives which are stated in Chapter 1 and addressed in the following chapters.

Genetic and cytological research in Chapters 3 and 4 revealed that the predominant Australian agricultural weed species of the genus *Salsola* is not *S. tragus* subsp. *tragus*, but rather *S. australis*. Further, other putative taxa of the genus *Salsola* that are closely related to *S. australis* are found in Western Australia and could not be matched to recognized species. These findings indicate that species of the genus *Salsola* may be native to Australia.

The biology and ecology of *S. australis* as investigated in agricultural environments of southwest Australia (Chapters 5, 6, and 7) were found to be similar to that of other

agricultural weed species of the genus *Salsola* in terms of growth habit, seed production, seed dispersal and seed bank characteristics. Total seed production of *S. australis* plants ranged from 138 to 7734 seeds and was correlated to plant biomass. Seed viability of *S. australis* was very low in several populations, which appeared to be due to genetic rather than environmental factors. The percent of the viable seed that germinated (established) in the year following seed production varied widely (from an average of 32.3 to 80.7%), and was influenced by the climate experienced by the maternal parent. The seed that shed easily from the plants was less likely to be dormant in the year following seed production than the seed retained on the plants.

Movement of *S. australis* plants in field conditions (1.6-1247.2 m) was not as widespread as that recorded for other species of the genus *Salsola* (Chapter 2 and 6), mainly because plant movement was restricted by entanglement with other *S. australis* plants in the population. Seed shedding resulted from plant movement, but was also related to the ageing and weathering of stationary or mobile plants. All plants retained a proportion of their seed, although germinability of retained seeds fell to less than 2% over two months.

Plants could establish and reach reproductive maturity and senescence throughout the year, although survivorship of cohorts varied according to time of establishment. However, *S. australis* was predominately restricted to a summer annual life cycle by the winter annual cropping system that is the most common land use in the southwest Australian wheat-belt. The cropping system halved total seed production of the *S. australis* population compared to non-cropped sites. *Salsola australis* plants do not appear to successfully compete within crops as is found for *S. tragus sensu lato* in broad scale dry-land cropping regions in the USA (Young 1986; 1988). While *S. australis* can grow within mature crops, it is not competitive in the early stages of crop development.

The data from Chapter 5, 6 and 7 was collated to construct a model (Chapter 8) in order to investigate the life cycle of *S. australis* in the broad scale cropping system at Lake Grace. Modelling *S. australis* at Lake Grace indicated that the population was not declining towards extinction, but rather had a positive growth rate, in spite of low seed viability (<15%). However, the model indicated that the number of viable seeds per plant significantly affected population growth rate, and so populations with high seed

viability may expand faster than the Lake Grace population. The model further demonstrated the importance of seed shed from immigrating mobile plants in maintaining annual seedling recruitment. The dormant soil seed bank had little impact on seedling recruitment. The model was used to assess possible control practices for use against this weed in the Western Australian agricultural system. Control practices that were most likely to be effective were those that targeted populations on a regional scale rather than a single field scale, or those that focussed on restriction of seed movement. As a result, physical control (i.e. removal of mature plants) caused the greatest reduction in population growth rate.

**Implications**

The genetic and cytological research has raised the possibility that some or all species of the genus *Salsola* in Australia are native plants. Bean (2007) developed a key for determination of origin status for non-endemic plant species in Australia, based on consideration of historical, ecological and phytogeographical criteria. In the case of *S. australis*, the historical evidence indicates that species of the *Salsola* genus in Australia predate European invasion (Bean 2007; Brown 1810; Smith et al. 1980). The ecological and phytogeographical data are equivocal and cannot be used to indicate the status of *S. australis*. However, *S. australis* was originally found in littoral habitats, growing on frontal sand dune systems and on the beach, above high tide mark (Bentham 1870; Brown 1810; Smith et al. 1980). There are many coastal species that are likely to have arrived in Australia prior to European invasion through long distance seed dispersal (Bean 2007; Groves and Burdon 1986; Kloot 1984). According to Bean (2007), a species such as *S. australis* that was in Australia prior to European invasion and grows in littoral habitats should be classified as indigenous, even though the ecological evidence for plant status is equivocal.

The discovery that *S. australis* is likely to be an indigenous species may influence the management of this species both in Australia and other countries in which it occurs, such as the USA and Africa (Rilke 1999a; Ryan and Ayres 2000) (Dr Fred Hrusa, pers. comm.). This was discussed in detail in Chapter 3 and 4. However, this research also affects the potential of *S. australis* to be of benefit in Australia. Native species are used by preference in the rehabilitation of mine sites and saline or degraded agricultural land. *Salsola australis* thrives in arid, degraded, saline regions (Mitchell and Wilcox 1988;

Naidu and Harwood 1997), and other species of the *Salsola* genus have proven to be useful in the revegetation of degraded regions in the USA and Central Asia (Toderich et al. 2002; Wali 1999). As an early successional species, *S. australis* establishes easily on disturbed sites and probably plays an important role in adding humus, improving soil structure and water infiltration, reducing erosion, minimizing raindrop impact and increasing soil microbe populations, thus improving the environment to allow establishment of other native species (Naidu and Harwood 1997).

Research into the population ecology of *S. australis* indicated the relative unimportance of the dormant soil seed bank. Seed likely remains dormant for short periods, resulting in emergence of multiple cohorts throughout the year (Chapter 5, Chapter 7). However, seed dispersal plays a more important role in maintaining population growth rate. Short lived dormant seed banks and broad scale seed dispersal are common traits for weed species of the *Salsola* genus (Chapter 2, Chapter 6). As a result, the importance of the mobile seed bank in maintaining annual seedling recruitment is likely to be as applicable for other weed species of the *Salsola* genus as it is for *S. australis*. Seed dispersal of *S. australis* is partially restricted by the wire mesh fences that are a standard feature of the Western Australian cropping system. However, in other broad scale cropping systems, such as those common in Washington State, pasture rotations are not common and fields within a farm or between farms are not always separated by fencing. In this type of system, prevention of seed dispersal of *Salsola* weed species may be difficult, but the model indicates that reduction of population size without restriction of seed dispersal is not feasible.

There are a number of other native and exotic species in Australia that form a structure which becomes mobile at senescence to disperse seeds. These include *Chloris truncate* R.Br., species of the *Agrostis* genus like *A. adamsonii* Vickery, *A. avenacea* J. F. Gmel. and *A. limitanea* J. Black, and *Nassella trichotoma* (Nees) Hack. ex Arechav. *Chloris truncate* is a native species that is becoming an increasingly prevalent weed in Western Australia, species of the *Agrostis* genus are endangered native plants (ANZECC 1999; Briggs and Leigh 1996; Brown 1997a; Cousens and Mortimer 1995) and *N. trichotoma* is an exotic weed of national significance (Australian Weeds Committee 2007). Methods of managing *S. australis* may be applicable to the management of these other mobile species, to reduce seed dispersal of the weeds and increase the colonization rate

of endangered native species. It is likely that the importance of seed dispersal, and the relative unimportance of the dormant seed bank will be constant traits between these species (Rees 1993). So the management options that improve or restrict seed movement are likely to have the largest impact on these other species.

The population ecology chapter confirmed that *S. australis* is predominately a summer weed within the southwest Australian cropping system. As was discussed previously, there has been limited research into the impact and control of summer weeds in Australia. Summer weed control poses unique challenges compared to winter weed control, as summer weeds become tolerant of herbicides due to the stressful conditions in which they grow (Mussell and Stewart 2004). Further research in this area will be required as summer weeds are likely to become an increasingly severe problem in Australia. Climate change predictions indicate that summer rainfall over much of Australia may increase, and autumn and winter rainfall will be reduced (CSIRO 2001; IPCC 2001). Increased summer rainfall would lead to increased population growth rate of summer weed species. Further, reduced autumn and winter rainfall would reduce the yield potential of the grain crops produced in the winter growing season. Crop yield is currently influenced by stored soil moisture from the summer fallow period (Tennant 2000), which is removed by the summer weed species (Osten et al. 2006). If winter rainfall is reduced, maximising crop yield will become increasingly dependant on stored soil moisture from the summer fallow. Therefore, the necessity of controlling summer weeds will increase, even as summer weed control becomes more difficult due to the increased weed growth rate.

**Future research**

While this thesis addressed each of the original research objectives, it has raised a number of issues that require further investigation. These are discussed in the prior chapters and summarised below.

- Research is required to investigate the genus *Salsola* throughout Australia, to determine the number and origins of species, and to determine if *S. tragus* or other exotic *Salsola* species are found in Australia.
- If species of the *Salsola* genus are native to Australia, then it is likely that they support biological control agents that will be of use in controlling *S. australis* and other weedy species of the *Salsola* genus that have invaded other continents.

- The potential of *S. australis* to be of benefit in Australia, in rehabilitation of degraded land, warrants further investigation.
- The biological data used to construct the model was predominately taken from populations of *S. australis* in a single district. The effect of changing key parameters of the model such as seed viability, to simulate the population growth rate of other populations of *S. australis* within the wheat-belt, would be beneficial.
- The effectiveness and practicality of physical or herbicidal management options for *S. australis* in field conditions need to be assessed. Further research is required to determine if biological control of *S. australis* in Australia is feasible, since this may be an indigenous species.
- The relationship between summer weeds and soil moisture levels, and the subsequent impact of summer weeds on both summer and winter crops in Australia needs to be investigated. This research will indicate the economic impact of summer weeds on the Australian cropping system, to determine the level of control that should be applied to these species during the summer fallow period.
- Further, research into summer weeds and soil moisture levels could be modelled and used to indicate the potential impact of climate change (i.e. lower winter rainfall, higher summer rainfall) on Australian cropping systems.

**Final comments**

*Salsola australis* is not a severe economic problem in Australian cropping systems when compared to other species of the *Salsola* genus in US agricultural systems (Young 1986; 1988). While *S. australis* can grow within mature crops, it is not competitive in the early stages of crop development. However, as a summer annual weed species, management of *S. australis* is still required to maximize subsequent crop yield potential. This thesis has covered some, but not all of the research that should be conducted on *S. australis*, and has indicated areas of future research required to improved management of the *Salsola* genus in Australia.

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# Appendix A: Voucher specimens discussed in Chapter 3 and 4

PERTH 07265239

*Salsola australis* R.Br.

Chenopodiaceae

Plant Description: Flowering to senescence.

Site Description: Roadside of farmland.

Locality: Brand Highway, 30 km S of Geraldton

State: WA

Lat: 28° 57' 3.600" S Long: 114° 45' 9.000" E (GDA94)

Collector: C. Borger 1

Collection Date: 14 March 2005

PERTH 07265409

*Salsola tragus* L.

Chenopodiaceae

Plant Description: Vegetative to dead (not mobile).

Vegetation: Native vegetation.

Site Description: Behind primary dunes.

Locality: Coronation Beach Road; Coronation Beach

State: WA

Lat: 28° 33' 6.100" S Long: 114° 33' 55.500" E (GDA94)

Collector: C. Borger 2

Collection Date: 14 March 2005

PERTH 07265395

*Salsola australis* R.Br.

Chenopodiaceae

Plant Description: Late flowering stage.

Site Description: Pasture field next to road.

Locality: Chapman Valley Road, 5 km E of Nabawa

State: WA

Lat: 28° 29' 49.700" S Long: 114° 48' 47.600" E (GDA94)

Collector: C. Borger 3

Collection Date: 14 March 2005

PERTH 07265387

*Salsola australis* R.Br.

Chenopodiaceae

Plant Description: Late flowering to senescence.

Site Description: Cropping field.

Locality: Corner Valentine Road and Nabawa Road E; Chapman Valley

State: WA

Lat: 28° 27' 35.600" S Long: 115° 6' 24.900" E (GDA94)

Collector: C. Borger 4

Collection Date: 14 March 2005

PERTH 07265360

*Salsola tragus* L.

Chenopodiaceae

Plant Description: Vegetative to flowering stage.

Site Description: Gravel roadside of farmland.

Other Notes: No other plants.

Locality: Mount Magnet Road; Geraldton

State: WA

Lat: 28° 41' 27.000" S Long: 115° 9' 3.300" E (GDA94)

Collector: C. Borger 6

Collection Date: 15 March 2005

PERTH 07265344

*Salsola tragus* L.

Chenopodiaceae

Plant Description: Early flowering or entirely dead and mobile.

Vegetation: Native vegetation.

Site Description: Road verge.

Locality: Great Northern Highway, 35 km N of Mount Magnet

State: WA

Lat: 27° 47' 14.900" S Long: 117° 55' 8.200" E (GDA94)

Collector: C. Borger 12

Collection Date: 15 March 2005

PERTH 07265352

*Salsola australis* R.Br.

Chenopodiaceae

Site Description: Roadside next to farm.

Locality: Mount Magnet Road; Geraldton

State: WA

Lat: 28° 37' 39.000" S Long: 115° 21' 21.600" E (GDA94)

Collector: C. Borger 7

Collection Date: 15 March 2005

PERTH 07265379

*Salsola tragus* L.

Chenopodiaceae

Plant Description: Vegetative to dead (not mobile)

Vegetation: Native vegetation.

Site Description: On primary dunes.

Locality: Broadhead Road; Greenough

State: WA

Lat: 28° 48' 5.300" S Long: 114° 37' 4.100" E (GDA94)

Collector: C. Borger 5

Collection Date: 15 March 2005

PERTH 07265492

*Salsola australis* R.Br.

Chenopodiaceae

Plant Description: Late flowering to senescence.

Vegetation: Native vegetation.

Site Description: Edge of field.

Locality: Gunyidi Road; Wubin

State: WA

Lat: 30° 7' 46.600" S Long: 116° 32' 43.400" E (GDA94)

Collector: C. Borger 15

Collection Date: 16 March 2005

Duplicates to: MEL

PERTH 07265476

*Salsola tragus* L.

Chenopodiaceae

Plant Description: Vegetative to senescence.

Vegetation: Coastal vegetation.

Site Description: Behind primary dunes.

Locality: Seaward Drive; Dandaragan

State: WA

Lat: 30° 17' 9.900" S Long: 115° 2' 44.700" E (GDA94)

Collector: C. Borger 17

Collection Date: 16 March 2005

Duplicates to: CANB

PERTH 07265484

*Salsola australis* R.Br.

Chenopodiaceae

Plant Description: Late flowering stage.

Vegetation: Native vegetation.

Site Description: Road verge.

Locality: 5 km W of Watheroo Road; Watheroo

State: WA

Lat: 30° 18' 52.900" S Long: 116° 1' 21.900" E (GDA94)

Collector: C. Borger 16

Collection Date: 16 March 2005

PERTH 07265336

*Salsola tragus* L.

Chenopodiaceae

Plant Description: Vegetative or dead.

Vegetation: Native vegetation.

Site Description: Road verge.
Locality: Great Northern Highway, N of Paynes Find
State: WA
Lat: 29° 3' 6.800" S Long: 117° 44' 46.600" E (GDA94)
Collector: C. Borger 13
Collection Date: 16 March 2005

PERTH 07265441
*Salsola australis* R.Br.
Chenopodiaceae
Plant Description: Late flowering to senescence.
Site Description: Field with crop stubble.
Locality: Corner Newdegate-Ravensthorpe Road and Old Ravensthorpe Road; Lake Grace
State: WA
Lat: 33° 5' 43.400" S Long: 119° 4' 3.400" E (GDA94)
Collector: C. Borger 20
Collection Date: 26 March 2005

PERTH 07265468
*Salsola tragus* L.
Chenopodiaceae
Plant Description: Vegetative to flowering stage [some plants just entering].
Vegetation: Trees and dead grass.
Site Description: Gravel roadside next to field.
Locality: 10 km E of Wagin on the Wagin-Dumbleyung Road; Wagin
State: WA
Lat: 33° 18' 8.600" S Long: 117° 23' 32.300" E (GDA94)
Collector: C. Borger 18
Collection Date: 26 March 2005

PERTH 07188307
*Salsola tragus* L.
Chenopodiaceae

Plant Description: All growth stages.

Site Description: Vacant block on edge of dune vegetation.

Locality: Seville Street just past Barcelona Drive; Cervantes

State: WA

Lat: 30° 30' 39.200" S Long: 115° 4' 8.400" E (GDA94)

Collector: C. Borger 21

Collection Date: 31 March 2005

Duplicates to: CANB

PERTH 07265255

*Salsola tragus* L.

Chenopodiaceae

Plant Description: Vegetative to dead stage.

Site Description: Vacant block on edge of dune vegetation.

Locality: Nambung National Park, 8 km S of Seville Street; Cervantes

State: WA

Lat: 30° 33' 50.800" S Long: 115° 6' 23.800" E (GDA94)

Collector: C. Borger 22

Collection Date: 31 March 2005

PERTH 07265433

*Salsola australis* R.Br.

Chenopodiaceae

Plant Description: Vegetative to mobile plants.

Site Description: Roadside of farmland.

Locality: Great Eastern Highway, 15 km W of Kellerberrin at the 15 KE sign; Tammin

State: WA

Lat: 31° 37' 56.300" S Long: 117° 34' 2.300" E (GDA94)

Collector: C. Borger 24

Collection Date: 7 April 2005

PERTH 07265247

*Salsola australis* R.Br.

Chenopodiaceae

Plant Description: Young plants and dead to mobile plants.

Site Description: Fenceline on farm.

Locality: Great Eastern Highway, 5 km W of Merredin

State: WA

Lat: 31° 30' 9.400" S Long: 118° 13' 39.700" E (GDA94)

Collector: C. Borger 25

Collection Date: 7 April 2005

# Appendix B

## Seed viability and dormancy in roly poly (*Salsola tragus* L.) populations

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**Summary** *Salsola tragus* is a summer annual weed found throughout Australia. The characteristics of the seed bank, including seed viability and dormancy, were investigated for populations from the south-west of Western Australia. Seed bank characteristics were investigated for seeds that are released from the mature plants as well as for seeds that remain attached. Average seed viability ranged from 40% to less than 2% for seed collected at plant maturity. Seed viability levels were the same for attached seed and released seed, but a higher proportion of attached seeds were dormant. The portion of dormant seed produced was also variable between populations, and was higher for plants in stressful environments.
**Keywords** *Salsola tragus*, seed viability, dormancy.

### INTRODUCTION

*Salsola tragus* L. (Chenopodiaceae) is a summer annual weed in agro-ecosystems in the south-west of Western Australia. The climate is mediterranean and summer rainfall is sporadic and very low on average. Summer annual weeds usually survive these conditions by producing and dispersing dormant seeds, which establish a viable soil seed bank (Salisbury 1961).

Seed dispersal ensures that seeds germinate in spatially separated environments. Seed dormancy ensures that seeds will grow in different years, in temporally separated environments. So both strategies ensure that the seedlings face heterogeneous environments and the likelihood of all seedlings facing an unsuitable environment is reduced (Fenner and Thompson 2005; Rees 1993).

Some species of the *Salsola* genus engage in broad seed dispersal (Mallory-Smith et al. 1993; Stallings et al. 1995). Following senescence, a specialised layer of cells at the base of the stem degrades, allowing the plant to break free of its roots (Young 1991). Pushed by the wind, the freed plants may move considerable distances (average of 3-4 km) (Stallings *et al.* 1995). Some seed is released from the plants easily and some seed is retained and gradually released through the tumbling motion of the plants (Stallings *et al.* 1995).

The key to control of *S. tragus* includes the management of both the soil seed bank, consisting of seeds that are released from the mature plants, and the mobile seed bank, consisting of the seeds that remain attached to the plant. The purpose of this study was to investigate seed viability and seed dormancy aspects of the seed bank of *S. tragus* populations being targeted for management in cropping environments of south-west of Western Australia.

### MATERIALS AND METHODS

**Seed source – UWA seed** Seed were harvested from field populations of *Salsola tragus* in the Shires of Morawa (S28°50.065' E115°46.580'), Merredin (S31°30.094' E118°13.397') and Lake Grace (S33°7.426' E118°28.592') in Autumn 2004. Seeds from each population were planted in 10 pots (194 mm diameter, 205 mm tall) filled with potting mix (50% mulched pine bark, 25% sand, 25% peat moss) on 20 January 2005 and placed in an outdoor netted enclosure at the University of Western Australia (UWA), Nedlands Campus. Seedlings were thinned to two plants per pot following establishment, and were fertilised and watered as required to ensure healthy growth. Populations were spatially separated to prevent pollen flow between them.

The Morawa population was harvested on 11 May 2005, the Lake Grace population on 30 May 2005 and the Merredin population on 7 June 2005. Plants were threshed using a Perspex thresher (Kingaroy Engineering Works Pty Ltd) to release seed. Despite threshing, some *S. tragus* seed remained attached to the parent plant following senescence. The threshed material from each population was sieved to separate the loose seeds from the seeds attached to plant material.

**Seed source – Field seed** *Salsola tragus* plants were harvested from the above sites in Autumn 2005. Plants were threshed and processed as for the UWA populations.

**Seed viability** Three samples of 100 loose and 100 attached seeds were removed from each field and UWA population. The samples were placed in petri dishes on wet filter paper. The petri dishes were sealed in plastic bags to retain moisture, and incubated in a growth cabinet with a 12 hr temperature cycle of 25/15°C. Germinated seeds were scored as viable and removed daily for 1 week. These seeds were classified as non-dormant. The remaining seeds were removed from the fruiting perianth and exposed to 1% 2,3,5-triphenyltetrazolium chloride (Sigma-Aldrich Co.) for 48 hr at 30°C (TZ test) (International Seed Testing Association 1999). Seeds containing embryos that turned purple were scored as viable. These seeds were classified as dormant.

The data were analysed using ANOVA. The proportion of non-dormant seed and dormant seed were compared for free and attached seed, within and between populations. Means that were significantly different were separated by LSD values.

### RESULTS

Total seed viability was highest in Morawa populations and in seed developed at UWA. Seed dormancy was higher for attached seed than loose seed, and higher in field seed than UWA seed (Table 1). Seed of the Morawa populations was significantly more viable than that of the Lake Grace and Merredin populations ($P_{0.05}$ <0.001, LSD: 2.47). The majority of seed produced by the Morawa populations was non-dormant. The percent of dormant seeds was significantly higher in seed from Lake Grace populations ($P_{0.05}$ = 0.036, LSD: 2.084).

For all three populations the attached seed had a higher proportion of dormant seeds than the loose seed samples ($P_{0.05}$ <0.001, LSD: 1.701). There was no significant difference between viability of attached and loose seeds.

The UWA plants generally produced seed with higher viability than the field plants, although the reverse was evident in the Lake Grace populations ($P_{0.05}$ <0.001, LSD: 1.191). However, field populations produced a higher proportion of dormant seed ($P_{0.05}$ = 0.023, LSD: 1.701). The Merredin field plants produced 100% dormant seed, while the UWA population produced an average of 29% dormant seed. The Lake Grace field and UWA populations produced an average of 88% and 46% dormant seed. The Morawa field and UWA populations both produced very little dormant seed (8% and 5%).

**Table 1.** Average viability of three replications of 100 loose and 100 attached seed for each population, grown in field conditions or at UWA. Total viable seed refers to the dormant and non-dormant seed.

| Population | Seed type | Field seed | | UWA seed | |
|---|---|---|---|---|---|
| | | Total viable seed | Dormant seed | Total viable seed | Dormant seed |
| Morawa | Attached | 43.67 | 4.00 | 77.00 | 5.33 |
| Lake Grace | Attached | 15.33 | 13.67 | 2.33 | 1.67 |
| Merredin | Attached | 1.33 | 1.33 | 26.00 | 10.00 |
| Morawa | Loose | 39.33 | 4.00 | 91.33 | 0.67 |
| Lake Grace | Loose | 9.33 | 8.00 | 3.33 | 0.67 |
| Merredin | Loose | 1.33 | 1.33 | 10.67 | 2.00 |
| LSD (P = 0.05) | | 4.94 | 4.17 | 4.94 | 4.17 |

## DISCUSSION

The proportion of dormant seed produced was very low for the Morawa populations. The bulk of the seed produced by Morawa populations germinated as soon as sufficient moisture was available and do not have sufficient dormancy to prevent germination until the following summer growing season. This suggests that these populations rely on seed dispersal rather than seed dormancy to ensure that the seed encounters sufficiently heterogeneous environments to allow some seeds to germinate and establish themselves successfully each year. The Lake Grace and Merredin populations often produced more dormant seed than non-dormant seed, and, therefore, are capable of establishing a seed bank. Clearly, reducing the seed bank through persistent, annual weed control will be more effective against *S. tragus* populations that do not establish long term seed banks.

Dormancy was higher for attached seeds than loose seeds. Attached seeds obviously require higher dormancy levels, as a seed that germinates while attached to the parent plant would have a very poor chance of survival (Wallace *et al.* 1968). Given that the attached seeds are equally viable and more likely to be dormant than the loose seeds, they may be as important to seedling recruitment as the released seeds in the soil seed bank. Therefore, the removal and destruction of mature plants is necessary to manage the seed bank.

The higher proportion of dormant seed produced by the field populations compared with the UWA populations was probably in response to the harsher environment experienced by field plants. Populations growing in the field may need a dormant seed bank to ensure survival, whereas a population growing at UWA in conditions with plentiful resources and no competition from other plants are less likely to require a dormant seed bank. So *S. tragus* plants may produce seeds adapted to the environment that the seeds are likely to encounter.

Seed viability was unusually low in all three populations, since other studies on seed viability of *S. tragus* (misclassified as *S. kali*) plants in the USA indicate that viability usually ranges from 80-100% (Evans and Young 1972; Ignaciuk and Lee 1980; Young 1991). Either environmental factors such as climate or pathogens adversely affected the seed viability, or a genetic factor such as altered ploidy levels was affecting viability.


## ACKNOWLEDGMENTS

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