# **Ecology and management of weeds under no-till in southern Australia**

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#### Abstract

No-till systems have been widely adopted by farmers in Australia over the past decade to reduce soil erosion, improve soil physical and chemical properties, conserve soil moisture and save on fuel costs. These changes in tillage practices can have a major influence on the ecology and management of weeds. Studies were undertaken on the seed biology of six important Australian weed species to provide underpinning knowledge of their response to tillage. Field studies were also undertaken to investigate the effect of no-till on weed seedling emergence, seed bank persistence and herbicide behaviour.

Seed germination of threehorn bedstraw and wild turnip, the latter only at suboptimal temperatures, was inhibited by light. In contrast, seed germination of common sowthistle and Indian hedge mustard was stimulated by light. Seed germination of small-flowered mallow was not influenced by the light conditions. Seedling emergence of threehorn bedstraw, wild turnip, small-flowered mallow and annual ryegrass was low on the soil surface but increased with shallow burial, which suggests that farming practices that achieve shallow burial of seeds are likely to promote greater seedling emergence of these weed species. In contrast, seedling emergence of common sowthistle and Indian hedge mustard was greatest for the seeds present on the soil surface and emergence decreased with increased burial depth.

In field experiments, low soil disturbance tillage systems left more seeds on the soil surface after crop sowing, whereas high soil disturbance systems buried most of the seeds. Seedling emergence of annual ryegrass, threehorn bedstraw and wild radish was greater under minimum tillage than no-till system. In contrast, seedling emergence of Indian hedge mustard, common sowthistle, silvergrass, small-flowered mallow and turnipweed was greater under the no-till system. Seedling emergence of wild oat and wild turnip was not influenced by the tillage system. Even though seedling emergence of annual ryegrass from one season to the next was similar between the tillage systems. This was because of much greater seed decay under no-till (48 to 60%) than that recorded under minimum tillage (12 to 39%).

All dinitroaniline herbicides (trifluralin, pendimethalin and oryzalin) were more effective in reducing the number of plants, spikes, dry matter and seed production of annual ryegrass when incorporated at sowing with tines than with the discs. At Minlaton in 2004 and 2005, bioavailable trifluralin was greater under tillage systems with greater levels of soil disturbance than under lower soil disturbance systems. In the absence of the herbicide, annual ryegrass was less competitive with wheat under the disc-sown systems. The response of grain yield to herbicides was greater under the tine-sown systems than the disc-sown systems.

The performance of S-metolachlor on annual ryegrass control was investigated under no-till. The control of annual ryegrass was greater than 80% when S-metolachlor was applied at sowing (incorporated by sowing or post-sowing pre-emergence). However, application of the herbicide at sowing resulted in phytotoxic effects on crop emergence and grain yield of wheat. Application of S-metolachlor at 20 or 23 days before sowing not only provided effective control (74 to 83%) of annual ryegrass, it was also safe on wheat. Application of this herbicide at 40 or 46 days before sowing was relatively ineffective in controlling annual ryegrass (33 to 49% weed kill) but safe on wheat.

In conclusion, soil disturbance caused by tillage was found to have a major influence on the behaviour of the seed bank of different species including seedling emergence and decay rates of weed seeds. However, the response to tillage tended to be speciesspecific and was related to their seed biology. Tillage systems also had a major influence on the efficacy and bioavailability of trifluralin, which is prone to volatilisation losses. The findings of this research program are expected to contribute to the improvement in weed management under no-till systems.

## Declaration

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

I give consent to this copy of my thesis, when deposited in the university libraries, being available for photocopying and loan.

Bhagirath Singh Chauhan

Date:

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# **Chapter 1. General introduction**

### **1.1 Background to the Study**

Reduced tillage systems have been widely adopted by growers in Australia over the past decade to reduce soil erosion, improve soil physical and chemical properties, conserve soil moisture and save on fuel costs. In 2001, more than 40% of the Australian cropping area was sown under no-till (NT) and over the next few years, this area is expected to increase (D'Emden and Llewellyn 2004). In addition to the soil and agronomic benefits, changes in tillage practices can also have a major influence on weed ecology including seedling emergence patterns of weed species in the field. Research from elsewhere in the world has demonstrated that weed seedling emergence responses to tillage systems are species-specific (e.g. Buhler and Daniel 1988). There has been considerable conjecture in Australia about the effect of NT on the relative abundance of weed species, but there is little published data available on this issue on important Australian weed species.

To improve management systems for specific weed species, it is critical to have good information on seed germination, persistence and seedling emergence (Mennan and Ngouajio 2006). Seed germination is a key event in determining the success of a weed in an agroecosystem, a process regulated by several environmental factors such as temperature, light, soil salinity, pH and moisture (e.g. Chachalis and Reddy 2000). These factors could affect seed germination of a weed species. Seed burial depth may also influence emergence of weed seedlings (Benvenuti et al. 2001; Boyd and Van Acker 2003) as these depths vary in availability of moisture, diurnal temperature fluctuation and light exposure. All these attributes of the microenvironment have the potential to influence the behaviour of weed seeds. Despite the growing concerns about threehorn bedstraw (*Galium tricornutum* Dandy), common sowthistle (*Sonchus oleraceus* L.), Indian hedge mustard (*Sisymbrium orientale* L.) and small-flowered mallow (*Malva parviflora* L.) in southern Australian farming systems, knowledge of their seed biology is limited.

Annual ryegrass (*Lolium rigidum* Gaud.) is a major weed of cropping systems in southern Australia and is generally recognised as one of worst weeds in the world as far as development of herbicide resistance is concerned. It has now evolved resistance to herbicides with many different modes of action (Heap 2004). In annual ryegrass, there is some evidence for lower seedling emergence under untilled than tilled systems (Peltzer and Matson 2002). However, the long-term fate of seeds that remain in the seed bank as a consequence of lower seedling emergence under NT seeding systems is not known. Do the seeds that fail to germinate under NT decay before the start of the next growing season or do they become part of a more persistent seed bank? The answers to these questions could have significant implications for weed management.

On farms, herbicides remain a major component in Australian crop production costs, estimated at \$38.80 per hectare per year for the grains industry (Sinden et al. 2004). This high expenditure on herbicides is an indication of the reliance of the Australian agricultural industry on herbicides as the principle method of weed management. Dinitroaniline herbicides, particularly trifluralin, are widely used to control annual ryegrass in southern Australia. However, the increasing use of NT crop establishment in recent years in Australia has changed the soil microenvironment for the performance of dinitroaniline herbicides, especially trifluralin.

Trifluralin is thought to dissipate primarily by photodecomposition and volatilisation (Grover et al. 1997), as it has a very high vapour pressure. Incorporation of trifluralin in soil can reduce volatilisation and photodegradation losses (Basham and Lavy 1987; Savage and Barrentine 1969; Walker and Bond 1977). On the other hand, the rate of loss can be rapid if the herbicide is not adequately incorporated into soil. As a result of the herbicide losses, there may be insufficient herbicide remaining to control the late emerging cohorts of annual ryegrass under NT systems. As grain growers move towards greater reductions in soil disturbance at sowing (e.g. discs), the rapid loss of trifluralin from soil is likely to become increasingly important. In addition, different NT seeding systems (e.g. discs and tines) are likely to provide different patterns of soil disturbance and this could influence herbicide incorporation and hence efficacy. At present, the information on soil disturbance effects on herbicide incorporation and efficacy under Australian growing conditions is limited.

As mentioned earlier, trifluralin is being widely used for the control of annual ryegrass in Australia; however, cases of resistance have been found against this herbicide (e.g. McAlister et al. 1995). With several more cases of trifluralin resistance discovered recently, there is a need to find an effective herbicide with a different mode of action. S-metolachlor could be one such herbicide.

S-metolachlor is a chloroacetamide herbicide, which is widely used in a wide variety of crops, such as corn (*Zea mays* L.) to control annual grasses and some annual broadleaf weeds (Mueller et al. 1999; Rouchaud et al. 1999). However in Australia, this herbicide is not recommended for annual ryegrass control in wheat (*Triticum aestivum* L.) due to phytotoxic effects on the crop when used at rates that are effective against annual ryegrass. It could be applied well before the sowing of wheat, so that it dissipates to a level that is safe for wheat but effective on annual ryegrass. However, the information on the effect of early pre-sowing application of S-metolachlor on the control of annual ryegrass in wheat is lacking.

The study entitled, "ecology and management of weeds under no-till in southern Australia" was designed with the following objectives:

(1) To study the factors affecting seed germination of important Australian weed species,

(2) To study the influence of tillage systems on the weed seedling emergence pattern of several important Australian weed species,

(3) To study the influence of tillage systems on the vertical seed distribution and seed bank persistence of annual ryegrass,

(4) To study the influence of tillage systems on the efficacy of dinitroaniline herbicides and bioavailability of trifluralin, and

(5) To study the effect of application timing and dose of S-metolachlor on the control of annual ryegrass in wheat.

The basic structure of the thesis is presented below:

Chapter 2 provides an extensive review of published studies on weed ecology, herbicide efficacy and persistence as influenced by tillage systems.

The factors affecting seed germination and seedling emergence of important Australian weed species are described in Chapter 3.

Chapter 4 describes the influence of tillage systems on the seedling emergence pattern of several important Australian weed species and their depth of emergence.

The influence of tillage systems on vertical seed distribution, seedling emergence pattern and seed bank persistence of annual ryegrass is described in Chapter 5.

Chapter 6 evaluates the influence of tillage systems and three dinitroaniline herbicides (trifluralin, pendimethalin and oryzalin) on the control and growth of annual ryegrass. This chapter also reports influence of tillage systems on bioavailable trifluralin in the soil.

Chapter 7 presents information on the influence of application timing and dose of Smetolachlor on annual ryegrass control in wheat.

Finally in Chapter 8, the results of the whole project are discussed in relation to the main objectives proposed (this Chapter) as well as the potential implications for weed management.

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# Chapter 2. Review of literature<sup>\*</sup>

#### 2.1 Abstract

In the last few years, there has been a growing trend towards reducing tillage in cropping systems so as to allow stubble retention, earlier planting and improved soil structure. However, the adoption of conservation tillage systems will change weed control practices. Different tillage systems interact with the microenvironment of weed seeds and can influence the pattern of emergence from the weed seed bank. Here, the literature on the effect of different tillage systems on weed ecology, herbicide activity and herbicide persistence has been reviewed. Tillage systems can have a major influence on the vertical distribution of weed seeds in the soil seed bank. However, the impact of the changes in the vertical seed distribution on weed seedling emergence is not well understood. Usually weed seedling emergence increases if tillage equipment brings buried seed to, or close to, the soil surface and seedling emergence decreases if surface seed is buried deeper in the soil. However, tillage responses have a tendency to be species-specific and can also be influenced by the intensity of tillage. Any weed species in which germination is stimulated by exposure to light is likely to become more prevalent under reduced tillage systems. Likewise, species that require burial for germination may become less prevalent. Few studies have investigated the fate of weed seeds that fail to germinate under any tillage system. Further research is needed to know whether the weed seeds that fail to germinate, decay before the start of the next growing season or become part of a persistent seed bank. Crop residues present on the soil surface can intercept a considerable amount of the applied herbicide and depending on the herbicide, this intercepted component is susceptible to losses. Therefore, conservation tillage systems are expected to have lower efficacy of soil active herbicides. However, there has been little investigation of rate of loss of soil active herbicides under reduced

<sup>\*</sup> The contents of this chapter have been accepted for publication.

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tillage systems and the results reported have been inconsistent. Much of the research on these effects is from overseas and may not fit in Australian conditions. Therefore, further work is needed to clearly understand the impact of changing tillage systems on weed ecology, herbicide activity and persistence.

## **2.2 Introduction**

Tillage involves soil disturbance with a variety of equipment to achieve different objectives, including weed control and seedbed preparation. However, tillage has been criticised as the main cause of soil erosion and a destroyer of soil structure (Hamblin 1987; The Parliament of the Commonwealth of Australia 1989). In Australia, acceptance of the evidence that severe tillage damaged soil structure was important for the development and adoption of conservation tillage systems. However, the concern over rising fuel costs was probably an even more important factor in the adoption of conservation tillage systems (Ellington 1979; Pratley and Rowell 1987).

Over the last two decades, there has been increasing emphasis on the need to reduce the amount of tillage to enhance the sustainability of the land. The benefits include reduction in soil erosion (Chan and Pratley 1998; Cogo et al. 1984; Griffith et al. 1986; Triplett and VanDoren 1977), fuel and labour savings (Frye 1984), increased soil moisture conservation (Unger 1981), improvement in soil tilth (Doran and Linn 1994), lower surface runoff of herbicides and fertilisers, better timeliness of sowing crops (Touchton and Johnson 1982; Unger 1981) and improved profits. In 2001, more than 40% of the Australian cropping area was sown under no-till (NT) and over the next few years, this area is expected to increased dramatically (ABARE 2003; D'Emden and Llewellyn 2004).

In Australia, several different types of tillage practices are employed; however, this has resulted in confusing definitions of tillage. Therefore, it is necessary to define different tillage practices before starting to review these systems. Conventional tillage (CONT) systems are those that leave the soil surface with little plant residue. Conservation tillage systems leave at least 30% of the soil surface covered with plant residue after planting (e.g. Buhler 1995). Lal (1995) defined conservation tillage as

"whatever sequence of tillage operations that reduces the losses of soil and water, when compared to conventional tillage". CONT systems are characterised by a number of tillage operations prior to sowing a crop and usually involve inversion of soil as the primary tillage operation followed by multiple trips with secondary tillage equipments. Direct drilling is a practice where the crop is sown without prior tillage, but where considerable soil disturbance occurs at seeding from the use of wide soil openers. Direct-drilled systems may also include some prior soil disturbance through pre-drilling fertiliser. NT is a practice where soil disturbance is minimised and the crop is sown with narrow soil openers or discs.

The presence of crop residues and lack of soil disturbance in reduced tillage systems have been found to increase the biological activity in the soil (House and Brust 1989). Different tillage systems will leave weed seed at different places in the soil profile. Furthermore, crop residues present under conservation tillage can also modify weed seedling emergence relative to the tilled areas. Conversely, effective weed management may become more difficult after the adoption of conservation tillage (Buhler et al. 1994; Clements et al. 1994; Kapustra 1979; Kapustra and Strieker 1976; Koskinen and McWhorter 1986; Robertson et al. 1976; Vincent et al. 1978; Wicks et al. 1972). Conservation tillage might influence the behaviour of soil active herbicides because residues retained under conservation tillage can intercept a large proportion of the applied herbicide and thereby reduce the amount of herbicide reaching the soil surface. This portion of herbicide might be more vulnerable to volatilisation, photodegradation or other losses, such as binding to residue. In Australia, there is a serious lack of pertinent information on the relationship between tillage, herbicide behaviour and weed biology of important weed species.

This chapter examines literature related to the effect of different tillage systems on weed ecology, herbicide activity and herbicide persistence.

### 2.3 Tillage System Effects on Weed Ecology

#### 2.3.1 Weed Seed Bank

Soil seed banks are reserves of viable seeds present in the soil and on its surface. The seed bank consists of both recent and older seeds shed in, and dispersed into, a region (Dekker 1999). Broadly, the seed bank can be classified into transient and persistent components (Thompson and Grime 1979). The transient seed bank is where the seeds produced in a given year do not remain viable for more than one year, whereas the persistent seed bank contain seeds that are one or more years old. Seeds can be dispersed into a region horizontally and vertically by wind, water, animal, agricultural implements or activities such as tillage. In this chapter, only the vertical seed distribution as influenced by tillage systems has been discussed.

Tillage has a major influence on the vertical distribution of seeds in arable soils (Cousens and Moss 1990; Roberts 1963; Staricka et al. 1990) and vertical seed distribution is a critical factor affecting seed survival, germination and emergence (Mohler 1993). Different tillage systems, based on their soil disturbance behaviour, redistribute weed seeds through the soil profile. In terms of the level of soil disturbance, tillage systems can be ranked as: NT < chisel plough (CP) < mouldboard plough (MB).

Pareja et al. (1985) found 85% of all weed seeds in the upper 5 cm of soil in a reduced tillage system, but only 28% seeds were found in this region in MB system. In a later study, 51% of the weed seeds were present within 4 cm of the surface in CP sown-plots compared to 11% in MB plots (Staricka et al. 1990). Yenish et al. (1992) found over 60% of the weed seed bank in the top 1 cm soil in NT system, but only 30% seeds were present at this depth in CP. In another study, more than 90% of weed seeds were found within 2 cm of the surface in NT system (Yenish et al. 1996).

In Australia, the term autumn tickle is used to describe a practice in which a shallow cultivation to a depth of 2 to 3 cm is given in the autumn season to improve seed-soil contact (Pearce and Holmes 1976). This practice also influences the vertical distribution of seeds in the soil and can increase the rate and amount of germination of annual ryegrass (*Lolium rigidum* Gaud.) seeds after autumn and early winter

rainfall events. Consequently, a greater proportion of weed seedlings can be killed by weed control practices prior to crop sowing (Gill and Holmes 1997; Pearce and Holmes 1976).

The literature appears to consistently show that less aggressive tillage systems such as NT concentrate weed seeds near the soil surface (e.g. Feldman et al. 1997; Torresen et al. 2003). However, tillage practices such as a one-off MB will bury weed seeds at depths from where seedlings cannot emerge and could be used as a tool in integrated weed management (Douglas and Peltzer 2004; Kettler et al. 2000).

It is quite clear from the literature that conservation tillage systems tend to concentrate weed seeds on or close to the soil surface. However, these studies are mainly from overseas and many of the tillage implements used in these studies create severe soil disturbance and bury seeds deeply. Such information is lacking in Australia, where tillage implements used tend to be less aggressive and most weed seeds are likely to be present on the soil surface at crop sowing. Furthermore, there are a variety of NT systems (discs and tines) currently being used in Australia. Vertical seed distribution patterns caused by these tillage systems needs evaluation. In addition, the impact of different disturbance levels on weed seedling emergence under Australian conditions is unclear.

Such information would enhance our understanding of the impact of tillage systems on weed seedling emergence from the seed bank and may assist in improving the effectiveness of weed management programs. The knowledge of movement of seeds by different tillage systems will facilitate the development of models that describe the behaviour of weed seed bank. The results obtained from vertical seed distribution by tillage systems could also be used to predict tillage effects on the distribution of pesticides incorporated into the soil.

Weed seeds persist in soil by developing resistance to predation and decay through pods or impermeable seed coats, through prolific seed production or seed dormancy. Usually, seeds lose their viability more rapidly near the soil surface, through germination and mortality, than if they are buried deeper in the soil (Mohler 1993). This may be due to more suitable germination conditions (Banting 1966) and more pathogen and predator activity near the surface (Taylorson 1970). Tillage systems may also influence persistence of the weed seed bank from one season to another. However, the measured persistence of the weed seed bank among different studies has tended to be inconsistent. For example, in Australia an early study on annual ryegrass seed bank indicated seed persistence level to be below 1% (McGowan 1970). However, in a more recent study in Western Australia, Peltzer and Matson (2002) reported 20 to 30% persistence of the annual ryegrass seed bank. Changing cropping practices between 1970 and 2002 could have influenced the size and position of the weed seed bank. It is also possible that increased cropping intensity over this period may have selected for increased dormancy in annual ryegrass populations. Such large differences between published studies warrants further research in this area including studies on the effect of tillage systems on seed bank persistence.

Generally, the persistence of seeds increases with depth; however, this effect is not universal. The persistence of some annual grasses with relatively large, short-lived seeds (e.g. rigid brome, *Bromus rigidus* Roth) was found to decrease with burial depth (Gleichsner and Appleby 1989). But survival of wild oat (*Avena fatua* L.) seeds that did not produce seedlings increased with burial depth where they tend to go into secondary dormancy (Banting 1966; Mohler 1993; Wilson 1972; Wilson and Cussans 1975).

Although some information on the influence of different tillage systems on the seed bank exists in the literature, it is very limited and almost entirely from overseas. There are a number of problems with translating such studies from overseas to Australian conditions. Frequently, the types of tillage systems are different as are soil characteristics, climate, weed species and management practices. All these factors can influence the behaviour of the soil seed bank.

#### 2.3.2 Weed Emergence

#### 2.3.2.1 Direct Effects of Tillage

The effect of tillage on weed emergence depends on many factors such as the amount, type, timing, speed and depth of the tillage equipment and the modification of the soil environment by tillage. Seedling establishment is also influenced by factors such as predation, seed dormancy, seed longevity, seed size and potential of a seedling to emerge from a given depth. These are all influenced by the position of the seed in the soil profile (Oegema and Fletcher 1972; Yenish et al. 1996). As a result of aggressive tillage burying seeds deeply, small-seeded species may not be able to emerge from greater depth. As an example, emergence of silvergrass [Vulpia bromides (L.) S.F. Gray] has been reported to be severely reduced at depths greater than 1 cm (Dillon and Forcella 1984). Forcella (1984) also reported greater abundance of silvergrass in direct-drilled crops than in conventionally tilled crops. On the other hand, wild oat populations, a large-seeded species, tend to increase more with pre-sowing cultivations than under practices that involve no or minimal disturbance such as direct drilling (Medd 1990). Some seedlings of this species can emerge even from a depth of 20 cm (Sharma and Vanden Born 1978). Generally, weed emergence increases if tillage brings deep buried seed to the surface and emergence decreases if the system buries surface seed deeper in the soil.

As different types of tillage equipments leave weed seeds at different depths, this differential distribution of the seeds in the soil profile has the potential to change seedling emergence and weed population dynamics (Buhler 1991; Harper 1957). Smith (1968) reported that the seedling emergence of barley grass (*Hordeum leporinum* Link.) was greatest for seeds buried at 1 cm (82%) and decreased for seeds buried at 3 cm (59%). Cheam (1987) also found greatest emergence of doublegee (*Emex australis* Steinh.) seedlings from seeds buried at 1 cm (36 to 48%) and decreased for seeds buried at 5 cm (10 to 16%). In a later study, Young and Cousens (1999) found a decrease in seedling emergence of wild radish (*Raphanus raphanistrum* L.) if seeds were buried below 4 cm. However, seedling emergence of common sowthistle (*Sonchus oleraceus* L.) was greatest from depths of 0 and 1 cm and no seedlings emerged from 5 and 10 cm depths (Widderick et al. 2002). Although these studies did not investigate tillage effects *per se*, such species-specific

response to burial could have a major impact on the responses of these species to tillage systems.

Tillage may increase germination of some seeds by mechanisms such as exposure of buried seed to light, aeration of soil, increased soil temperature, removal of plant canopy and release of soil-bound volatile inhibitors. Tillage may also increase germination of some seeds by increasing soil-seed contact. In Australia, a shallow cultivation in autumn (known as an autumn tickle) is aimed at improving soil-seed contact to obtain faster and more uniform germination of weed seeds in order to maximise the pre-sowing weed kill (Gill and Holmes 1997; Medd 1987). However, the success of this technique is largely dependent on rainfall received after the initial cultivation (Morgan 1988). In Western Australia, Pearce and Holmes (1976) reported 30 to 43% increase in annual ryegrass seedling emergence following an autumn cultivation.

Tillage allows greater diffusion of oxygen into and carbon dioxide out of the soil, increases the amplitude of temperature fluctuations and favours nitrogen mineralisation (Mohler 1993). All of these factors are known to alleviate dormancy and stimulate germination. Tillage may also change soil properties resulting in varied weed emergence. For example, Cussans et al. (1996) found that emergence of weed species varied in response to clod size.

Froud-Williams et al. (1981) predicted that wind-disseminated species, annual and perennial grasses, perennial dicot species and volunteer crops would increase and annual dicot weeds would decrease with reduced-tillage. Association of perennial weed populations with reduced-tillage systems could be due to the lack of disturbance of the root system of established perennial plants (Triplett 1985). Later, Derksen et al. (1993) also found that wind-dispersed species and volunteer crops were associated with reduced tillage and summer annual dicots with CONT. They also concluded that an increase in perennial species was not always associated with reduced tillage but also by other agronomic practices employed in the cropping system. Increased abundance of wind-dispersed species under reduced tillage might have been due to the lack of seed burial by tillage equipment (Derksen et al. 1993; Froud-Williams et

al. 1981). Recently in an Australian study, emergence of common sowthistle was found to be greater under a zero tillage system than for systems with more severe soil disturbance (Widderick et al. 2004).

Reeves et al. (1981) showed strong stimulation of wild radish emergence by tillage. Similarly, Pratley (1995) reported greater density of fumaria (Fumaria spp.) where pre-sowing tillage was given. In another Australian study, wild radish numbers were significantly lower under MB compared with direct drilling (Code and Donaldson 1996). However, deeply buried seeds of wild radish have been found to persist for longer in the seed bank than seeds on or near the soil surface (Code et al. 1987; Reeves et al. 1981) and may cause reinfestation if brought to the surface by subsequent tillage operations. In a recent study, cultivation (up to 5 cm) was found to stimulate seedling emergence in annual ryegrass, wild oats, wall fumitory (Fumaria *muralis* Sond. Ex Koch) and wild radish, but not in barley grass (Peltzer and Matson 2002). The seedling emergence of catchweed bedstraw (Galium aparine L.) and false cleavers (G. spurium L.) was stimulated by tillage in overseas studies (Froud-Williams 1985; Reid and Van Acker 2005). Ogg and Dawson (1984) reported that emergence of four broadleaf weed species was stimulated, three broadleaf species was unaffected, and one grass species was inhibited by tillage. Similarly Popay et al. (1994), at the end of their 7-year study, reported a 7-fold greater total seedling emergence in deep-cultivated plots (28000/m<sup>2</sup>) and a 2.75-fold greater emergence in shallow-cultivated plots (11000/m<sup>2</sup>) when compared to uncultivated plots (4000/m<sup>2</sup>).

Tillage systems influence the periodicity of weed emergence in a more complicated manner because tillage affects both vertical seed distribution and the soil conditions surrounding the seed and there can be an interaction between these effects. In a study on velvetleaf (*Abutilon theophrasti* Medik.) and giant foxtail (*Setaria faberi* Herrm.), densities were counted in the untreated plots under MB, CP and NT system at 14, 28, 42, 56 and 77 days after corn (*Zea mays* L.) planting (Buhler and Daniel 1988). Velvetleaf densities in MB were greater than those in CP and NT at all observation dates. On the other hand, giant foxtail densities in CP and NT were greater than densities in MB at all observation dates. Recently, Bullied et al. (2003) reported that conservation tillage promoted earlier emergence than CONT for wild oat, fat hen (*Chenopodium album* L.) and green foxtail [*Setaria viridis* (L.) Beauv.].

Generally weed seeds present near the soil surface germinate and emerge earlier than seeds buried at deeper depth, because conditions for germination are more suitable near the surface and also seeds have to emerge or elongate over a shorter distance to come to the surface (Oryokot et al. 1997). However, slower seedling emergence could also be expected under NT as most of the seeds are present on the soil surface and are more prone to adverse conditions. This could mean that pre-sowing selective herbicides, such as trifluralin that are volatile and prone to photodegradation, may be less effective. In addition, late emerging weeds are likely to be less competitive with the crop and not have a substantial impact on crop yield loss and weed seed production. Plants emerging earlier can produce a higher number of seeds than later emerging ones (Cheam 1986; Fernandez-Quintanilla et al. 1986; Mack and Pyke 1983; Mohler and Calloway 1995). Mohler and Calloway (1995) reported that the majority of seeds of redroot amaranth (Amaranthus retroflex L.), fat hen, pigweed (Portulaca oleracea L.) and crabgrass (Digitaria sanguinalis L.) were all produced by the first cohort of plants to emerge. This difference in seed production can be an important feature of a tillage system. However, detailed data on important weeds of Australian cropping is generally lacking in the literature.

In the absence of research data in Australia, there has been considerable conjecture about the effect of NT on the relative abundance of weed species. Tillage responses tend to be species specific; however, the intensity of the tillage system can also be important. The rapidly changing seeding systems in Australia, typified by the rapid adoption of NT seeding systems, may result in dramatic changes in problematic weed flora. Therefore, there is a need to understand the seedling emergence ecology of weeds under changing tillage systems.

As described earlier, seedling emergence of some weed species was found to be lower under NT fields as compared to tilled fields. Such an outcome raises an important question: what is the long-term fate of the seeds that remain in the seed bank as a consequence of lower seedling emergence? Do the weed seeds that fail to germinate under NT decay before the start of the next growing season or do they become part of a more persistent seed bank? The answers to these questions could have significant practical implications for weed management and need addressing.

### 2.3.2.2 Light Effects

Different weed species have different responses to light and darkness for germination. It has been known for a long time that soil tillage in the presence of light stimulates the germination of more seeds than tillage without light (Sauer and Struik 1964; Wesson and Wareing 1969). Exposure to light breaks dormancy and eventually increases germination in many species, especially small-seeded species (Bouwmeester and Karssen 1989; Cousens et al. 1993). A species showing preference for light in germination has the potential to be more prevalent in NT systems and pastures (Cousens et al. 1993).

Many field experiments have been conducted to investigate the effects of night versus day tillage on weed seedling emergence (Botto et al. 1998; Buhler 1997; Gallagher and Cardina 1998; Hartmann and Nezadal 1990; Milberg et al. 1996; Scopel et al. 1994; Stockings et al. 2002; Van Ryswyk et al. 2004). Wesson and Wareing (1969) reported 62% greater emergence of dicot species when the soil was disturbed in the light than in the dark. Similarly, Hartmann and Nezadal (1990) reported up to an 80% reduction in weed emergence in nighttime tillage treatment as compared to daytime. The major species reduced after the nighttime tillage in this study were catchweed bedstraw, creeping speedwell (Veronica persica Poir.), wild chamomile (Matricaria chamomilla L.), penny cress (Thlaspi arvense L.) and field bindweed (Convolvulus arvensis L.). In another study, nighttime tillage reduced redroot amaranth and black nightshade (Solanum nigrum L.) emergence to one-fifth that of daytime tillage (Scopel et al. 1994). Similarly, Botto et al. (1998) reported a more than 200% increase in weed emergence in the plots tilled during the daytime with a MB than plots tilled at night. In a recent Australian study, Van Ryswyk et al. (2004) showed some delay in the emergence of bladder ketmia (*Hibiscus trionum* L.) and amaranth (Amaranthus sp.) following night soil cultivation and sowing.

Generally, small-seeded species are found to be more sensitive to light than largeseeded ones. For small-seeded broadleaf species such as fat hen, eastern black nighshade (*Solanum ptycanthum* Dun.) and Pennsylvania smartweed (*Polygonum pensylvanicum* L.), emergence was found to be lower when tillage was conducted during darkness than with tillage during the light (Buhler 1997). However, this tendency for small-seeded species to be more light sensitive than large-seeded ones was not observed by Milberg et al. (1996). Their data suggest that night tillage can reduce the emergence of some weed species due to their germination requirement for light and this approach could be used for weed management. However, the difference between daytime and nighttime tillage depends on the agricultural history of the field, the tillage implements used, vertical distribution of seeds, the time of the year and the species present in the field (Botto et al. 1998).

Usually daytime tillage stimulates the germination of weed seeds; however, high seedling emergence may occur in the nighttime cultivated fields (Ascard 1994; Buhler 1997; Jensen 1992; Scopel et al. 1994). Two possible reasons were given by Botto et al. (1998) for this observation. Firstly, hydrated seeds located close to the soil surface may be triggered to germinate by photons absorbed. Secondly, soil tillage may change the soil environment such as temperature, aeration and compaction, which could be responsible for stimulating germination even in the absence of light.

Some species have seeds that are inhibited from germinating in the light. For example, germination of false cleavers is strongly inhibited by light (Malik and Vanden Born 1987). This means that seeds left on the soil surface may not germinate until burial by the seeding operation. On the other hand, dormancy can be induced in light-requiring seeds even by shallow burial (Pons 1989; Wesson and Wareing 1969), since less than 1% of incident light can penetrate further than 2.2 mm into soil (Egley 1986; Wooley and Stroller 1978). Conversely, tillage can promote germination and emergence of some species, such as wild radish, because germination of these species could be stimulated by darkness and the availability of soil moisture surrounding the seeds (reviewed by Cheam and Code 1995).

Light exposure can have a large influence on weed germination. Previous research on annual ryegrass identified a 'dark dormant' component of the seed bank that only germinated when exposed to light (Gramshaw and Stern 1977). In a later study, Steadman (2004) also reported that dormancy release of annual ryegrass during darkstratification caused a gradual increase in sensitivity to light. Therefore, tillage can promote germination of some weed seeds due to exposure of dark-dormant component of seed bank to light. However, light responses are also species-specific. Eliminating light penetration during tillage can help in reduction of emergence of buried light-sensitive species. This information can be used as a component of weed management; however, information on this issue is limited on major weeds in Australia.

Light is known to stimulate germination of some weeds such as paradoxa grass (*Phalaris paradoxa* L.) (Taylor et al. 2004) and annual ryegrass (Gramshaw 1976), but research on other weeds such as threehorn bedstraw (*Galium tricornutum* Dandy) and Indian hedge mustard (*Sisymbrium orientale* L.) is lacking. Research is needed to understand the effect of daytime and nighttime tillage systems on important Australian weeds. It would be possible for farmers to adjust the timing of tillage and sowing with knowledge about the light response of weed species present in their fields. In Australia, some farmers already cultivate at night in order to cover more area and may be unwittingly changing the germination patterns of several weeds by doing so.

### 2.3.2.3 Seed Coat Effects

Dormancy can be imposed upon weed seeds by the coat itself, which is called physical or coat-imposed dormancy (Turner et al. 2005). Hard seededness is a relatively absolute form of coat-imposed dormancy due to the impermeability of covering structures to water and/or gases (Foley 2001) which is found in many weeds, including velvetleaf (Cardina and Sparrow 1997) and spiny sida (*Sida spinosa* L.) (Egley et al. 1986). Hard seeds generally require physical or chemical scarification, boiling in water, or stratification or weathering in the soil to enhance germination (Foley 2001). Seed covering structures and dormancy in hard seeds has been reviewed in detail by Egley (1989) and Kelly et al. (1992). Seeds of some species, such as small-flowered mallow (*Malva parviflora* L.) and round-leaved mallow (*M. pusilla* Sm.), have hard seed coats that need to be scarified to stimulate germination (Chorbadjian and Kogan 2004; Makowski and Morrison 1989). Due to impermeable seed coats, these seeds may have a long life in the soil (Egley and Chandler 1983).

In addition to impermeable seed coats, dormancy in some weed species, such as wild radish, is largely due to the pod surrounding the seeds (Cheam 1986; Mekenian and Willemsen 1975). Therefore, any mechanism that can increase pod breakdown in these types of seeds will result in an increase in germination and emergence (Young and Cousens 1999). As described earlier, impermeability to water is the most common factor involved in this type of dormancy.

Tillage is one factor that can stimulate germination by seed coat scarification. This could occur through direct contact with the tillage implement or through abrasion from moving soil particles. However, scarification during tillage is only likely to be a small component of germination stimulation as most of the seeds are likely to escape scarification during any given tillage operation. On the other hand, scarification on the soil surface can occur through trampling by hoofed animals, incomplete predation by animals or fungi, passage through an animal's digestive tract, and extreme changes in temperature and moisture regimes. Although these factors are not directly related to tillage systems, NT systems leave most of the seeds on the soil surface where they are more susceptible to such effects. Anecdotal evidence supports this through field observations of greater densities of certain weeds, such as small-flowered mallow, under NT. However, this response could also be due to tolerance to glyphosate (a non-selective herbicide), which is being widely used under NT systems. The role of scarification on weed seed germination and seedling emergence require additional experimental investigation in order to fully understand the effects.

## 2.4 Tillage System Effects on Herbicide Activity and Persistence

#### 2.4.1 Herbicide Activity

Changing tillage practices can also change herbicide performance, particularly for soil active herbicides. Herbicide performance can be influenced by the amount of soil disturbance, the degree of incorporation of the herbicide, the position of weed seeds in the soil and the amount of plant residue present. There has been considerable research on the interactions of tillage and herbicide performance, mostly from overseas. There has been very little research on this topic in Australia. In an overseas study, control of giant foxtail with atrazine, cyanazine and their mixture was lower in NT as compared to MB (Buhler and Daniel 1988). The high density of this weed in NT was reported to be responsible for the reduced control. In contrast, velvetleaf control was greater in NT than MB with the same herbicides. In a similar study, green foxtail and redroot amaranth were more difficult to control in CP and NT than in MB and ridge tillage (RT) systems with several herbicides (Buhler 1992). In an Australian study, trifluralin was found to be more effective for controlling rigid brome in direct-drilled plots as compared to the plots that received 1 to 3 tillage operations (Matic and Black 1990). Other studies have shown that some herbicide combinations that provide effective weed control in corn in tilled plots were not as effective in NT (Buhler and Daniel 1988; Triplett and Lytle 1972).

The efficacy of different herbicide formulations can also be influenced by tillage systems. For example, pre-emergence herbicides applied as granules (alachlor, cyanazine and metolachlor) were reported to provide better weed control than liquid formulations in NT, but differences between herbicide formulations were not evident under MB, CP or RT systems (Johnson et al. 1989). This difference between formulations under NT was due to low rainfall in that year and as a result the surface residue might have absorbed most of the liquid-formulated herbicides. As the effect of tillage systems on weed control varies with species and herbicides used, choosing an appropriate herbicide and application timing is critical in conservation tillage systems (Buhler 1995).

The presence of a greater amount of crop residue under conservation tillage as compared to more aggressive soil disturbance tillage systems, can affect the activity of herbicides used. However, the level of herbicide by residue interaction is hard to evaluate because of variable distribution of residue over the soil. Organic matter has been considered the principal soil component influencing weed control performance of most soil active herbicides (Hill et al. 1955; Peter and Weber 1985; Sheets 1958; Upchurch and Mason 1962). Soil organic matter seems to be more effective in inactivating herbicides with low water solubilities (Harrison and Weber 1975; Lamberts 1967; Leopold et al. 1960). Crop residues can intercept from 15 to 80% of the applied herbicide and this might be responsible for the reduced efficacy of some herbicides in conservation tillage systems (Banks and Robinson 1982, 1984; Buhler

1995; Ghadiri et al. 1984; Isensee and Sadeghi 1994; Sadeghi et al. 1998; Sorenson et al. 1991; Streit et al. 2003). However, in some situations presence of crop residue does not have a negative effect on herbicide efficacy (Crutchfield et al. 1986; Johnson et al. 1989; Worsham 1984). For example, weed control with alachlor plus atrazine was not influenced by surface residue unless herbicides were applied at lower than recommended rates (Erbach and Lovely 1975).

As NT systems leave most of the weed seeds on the soil surface, seedlings emerging on or near the soil surface may have little chance to contact shoot-absorbed herbicides and may not be controlled effectively by such herbicides (Prendeville et al. 1967). Herbicide placement can also be important in crop selectivity. Selective weed control can be achieved by sowing the crop below the herbicide band, provided the herbicide has limited soil mobility. Tillage, by distributing herbicides, can affect safety to the crop. For example in Australia, trifluralin use in NT wheat (*Triticum aestivum* L.) is considered much safer than in CONT wheat. The safe use of these herbicides within a NT system relies on the ability of the soil openers to move the chemically treated soil out of the crop row. Therefore, the type of soil opener, whether tine or disc, may also be important in herbicide performance.

Although some information about tillage system effects on herbicide activity exists in the literature, it is fairly limited and almost entirely from overseas. Australian farmers are still uncertain about issues of herbicide performance under reduced tillage systems. There is also uncertainty about the level of incorporation and movement of herbicides under NT. Further research is needed to clearly understand the effect of changing tillage systems on herbicide performance, as the activity of some of the soil active herbicides depends on the amount and pattern of soil movement by the tillage system.

#### 2.4.2 Herbicide Persistence

As discussed in the previous section, crop residues present on the soil surface under reduced tillage systems can intercept a significant part of the herbicide applied. For some herbicides such as trifluralin, this intercepted component is vulnerable to volatilisation, photodegradation and other losses (Grover et al. 1997). Such herbicide losses may be influenced by the amount of ground covered by crop residue, the herbicide chemical properties and formulation (Johnson et al. 1989). Incorporation of volatile herbicides in the soil can reduce volatilisation and photodegradation losses (Basham and Lavy 1987; Savage and Barrentine 1969; Walker and Bond 1977). However, the level of incorporation will differ markedly between different NT seeding implements. In contrast, the degradation rate for herbicides such as simazine and atrazine, which are less volatile and susceptible to photodegradation than trifluralin, was not found to be influenced by the application method (Buchanan and Hiltbold 1973; Hiltbold 1974). Depending on climatic conditions, herbicide application methods such as pre-plant incorporation or post-sowing pre-emergence, can strongly affect herbicide persistence (Curran et al. 1992).

The greater organic matter of soil in NT systems, a result of greater stubble retention, compared to CONT systems might increase the degree of herbicide protection from degradation (Locke and Bryson 1997). In contrast, for some herbicides retention by organic residues and soil components at the soil surface can increase losses due to volatilisation, photodecomposition, or other processes and reduce persistence (Jones et al. 1990; Mills et al. 1989; Thelen et al. 1988). For example, oryzalin was found to dissipate more rapidly in wheat straw covered soil than in non-mulched soil (Banks and Robinson 1984).

Photodegradation can be a major contributor to herbicide dissipation in the field, particularly under prolonged periods without rainfall when the herbicide remains on the surface of the soil or plant residue. Movement of herbicide off the plant residue and into the soil is important to facilitate absorption by weeds. This can only take place with rainfall or tillage under the rainfed agriculture practiced in southern Australia. As an example, photodecomposition of trifluralin occurs when herbicide on the soil surface is exposed to unfiltered sunlight (Wright and Warren 1965). In a laboratory study, photodecomposition losses after 7 days for pendimethalin, trifluralin and oryzalin were reported to be 9.9, 18.4 and 26.6%, respectively, (Parochetti and Dec 1978). Therefore, different levels of stubble cover and burial created by different tillage implements could influence the persistence of herbicides.

Herbicides with high vapour pressure are prone to volatilisation loss from the soil surface. Volatilisation of trifluralin (having high vapour pressure) from soil has been shown to be an important mode of loss (Bardsley et al. 1968; Savage and Barrentine 1969). Only 2% of the initial trifluralin amount was found after 12 weeks when the herbicide was not incorporated and volatilisation increased with increasing temperature (Savage and Barrentine 1969). Trifluralin has also been reported to be less persistent in soil after shallow incorporation than with deep incorporation (Oliver and Frans 1968; Savage and Barrentine 1969). In a later study, Kennedy and Talbert (1977) also reported that dinitroaniline herbicide persistence in soil depended upon the length of time before incorporation and the volatility of the herbicide. In this study, greatest losses and least persistence occurred when incorporation was delayed by 7 days. Similarly, pendimethalin was more persistent in the field when the herbicide was incorporated (80% remaining after 20 weeks) than when it was surface applied (20% remaining) probably because of lower volatilisation and photodecomposition of the chemical (Walker and Bond 1977). Therefore, dinitroaniline herbicides should be incorporated into soil within a few hours after application to prevent major loss of herbicide activity (Bardsley et al. 1968; Savage and Barrentine 1969; Weber 1990). Most of the studies on trifluralin persistence are from overseas, where temperature, moisture and soil properties are different. One study conducted in Australia showed trifluralin persistence varied with soil type and climate, persisting longer at a site with lighter soils and a drier climate (Johnstone et al. 1998). These studies were conducted under CONT systems and the persistence may be much lower in systems with less tillage.

Some herbicides are degraded mainly through microbial activity. As microbial populations have been found to be greater in NT systems (Doran 1980), these herbicides may dissipate faster in NT systems. In a dissipation study of several acetanilides, microbial degradation was found to be more important than volatilisation, chemical degradation and leaching (Zimdahl and Clark 1982). Also, microbial degradation was found to be the primary factor responsible for fluometuron and metolachlor decomposition in soil (Beestman and Deming 1974; Mueller et al. 1992; Yu et al. 1975). Therefore, metolachlor is more likely to degrade faster in NT systems due to microbial degradation than with more aggressive soil disturbance.

Usually herbicide persistence is found to be lower in NT systems than tilled systems. For example, Slack et al. (1978) found lower persistence of simazine under NT than CONT. Isensee and Sadeghi (1994) found an average of 2.6 times more atrazine in the surface 10 cm of soil under CONT than under NT. More leaching under NT via macropores was thought to be responsible for lower atrazine concentration in the surface layer under NT. For imazaquin and imazethapyr, no differences in herbicide concentrations in the surface layer of soil between CONT and reduced tillage system were reported (Curran et al. 1992).

The above studies are from overseas, as little work has been done in Australia on persistence of herbicides under different tillage systems. Amongst soil active herbicides, trifluralin is a widely used herbicide for the control of weeds in NT grain crops in Australia. Trifluralin is principally a root-absorbed herbicide that requires incorporation into the soil to be effective (Parka and Soper 1977). The significant increase in NT crop establishment that has occurred in recent years in Australia has changed the environment in which trifluralin has to perform. Grain growers have responded to the problems of reduced soil incorporation and increased crop residue on the surface by increasing the rate of herbicide applied. As grain farmers move towards greater reductions in soil disturbance at sowing, rapid loss of trifluralin from the soil is likely to become increasingly important. Herbicide losses of high magnitude could have serious implications for the control of weeds that show a staggered germination pattern. There may be insufficient trifluralin remaining to control late emerging weeds in crops. As the amount of soil disturbance decreases, the level of control offered by trifluralin can be expected to decrease. In very low disturbance systems trifluralin is unlikely to persist long enough to provide control of later emerging cohorts of weeds. However, as there are no published studies on the persistence of trifluralin under NT, there is an urgent need for information in this area.

#### **2.5 Conclusions**

There are a significant number of implications of changing tillage systems for weed control. Traditionally, tillage has been used as a primary means of weed control; however, concern about fuel and labour cost, soil structure and soil organic matter contents have led to cropping systems that have much lower levels of tillage. This development has been particularly important in Australia where there is increasing adoption of NT (D'Emden and Llewellyn 2004). As tillage is reduced, there is decreased soil disturbance, increased crop residue and increased reliance on herbicides for weed control. All three of these factors can influence the behaviour and control of weeds. In addition, there are many interactions that are, on the whole, poorly understood. As the amount of tillage continues to decrease, better understanding of these issues will enable better decision making in weed management.

Tillage systems can have a large effect on the vertical distribution of weed seeds. By recognising that the vertical distribution of seeds is a critical factor affecting seed survival, germination and emergence, we can solve much of the difficulty in understanding the effects of tillage on weed density (Mohler 1993). Tillage systems can also influence patterns of weed seedling emergence, but these effects tend to be species-specific and can be modified by the characteristics of the tillage system. Later emerging weeds are likely to be less competitive with the crop and may not have a substantial impact on crop yield loss and weed seed production.

Tillage systems play an important role in regulating seed bank behaviour by mixing weed seeds in the soil profile and affecting persistence of the seeds. Most of the seeds stay on the soil surface under NT and are more prone to unfavourable conditions and more likely to decay. Tillage also influences weed seedling emergence by affecting the light exposure of the seed bank; however, light responses are also found to be species-specific. Lastly, the amount of tillage can affect the efficacy and persistence of soil active herbicides, particularly herbicides like trifluralin that are volatile. Many tillage implements including discs have not been studied for performance under different travel speeds, angles and depths even for a single crop. Such data is needed if we are to predict the behaviour of weeds and herbicides in emerging tillage systems. Plant residue present on the soil surface under conservation tillage can intercept and play a role in the deactivation of a significant amount of herbicide under conservation tillage interact to influence weed ecology and herbicide persistence.
## **2.6 References**

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# Chapter 3. Factors affecting seed germination and seedling emergence of six Australian weed species<sup>\*</sup>

# **3.1 Introduction**

To improve management of different weed species, it is critical to have good information on seed germination, persistence and seedling emergence (Mennan and Ngouajio 2006). However, there is little information in the literature on the seed biology of important Australian weed species, making it difficult to develop effective weed management strategies.

Threehorn bedstraw (*Galium tricornutum* Dandy) is an important dicotyledonous weed of winter crops in southern Australia. It was assigned a declared weed status in Western Australia where it was recently introduced inadvertently (Department of Agriculture, Western Australia 2005). This means that the movement of machinery from contaminated fields is prohibited unless that machinery is thoroughly cleaned. In addition, any plants found must be destroyed. Threehorn bedstraw is also present in other states of Australia, including South Australia and the Wimmera region of

<sup>\*</sup> Some contents of this chapter have been published or accepted for publication.

Chauhan, B.S., Gill, G. and Preston, C. 2006. Factors affecting seed germination of threehorn bedstraw (*Galium tricornutum*) in Australia. Weed Science 54: 471-477.

Chauhan, B.S., Gill, G. and Preston, C. 2006. Seed germination and seedling emergence of threehorn bedstraw (*Galium tricornutum*). Weed Science 54: 867-872.

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Chauhan, B.S., Gill, G. and Preston, C. 2006. Influence of environmental factors on seed germination and seedling emergence of rigid ryegrass (*Lolium rigidum*). Weed Science (in press, Nov-Dec issue).

Victoria, where it can be a troublesome weed in wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), field peas (*Pisum sativum* L.), chickpeas (*Cicer arietinum* L.) and faba beans (*Vicia faba* L.) (Amor and Kloot 1987; Black et al. 1994; Moerkerk 1999).

There is little information on the ecology of threehorn bedstraw in the literature, making it difficult to develop strategies for the management of this weed. However, two closely related species, catchweed bedstraw (*G. aparine* L.) and false cleavers (*G. spurium* L.), have been well studied overseas (Froud-Williams 1985; Malik and Vanden Born 1988; Mennan 2003; Taylor 1999). In the absence of information about threehorn bedstraw, some insights can be gained from these closely related species.

Catchweed bedstraw can cause a significant yield loss in crops. For example, Peters (1984) reported yield losses from winter wheat of 30, 47 and 52% at densities of 25, 100 and 520 catchweed bedstraw plants/m<sup>2</sup>. This species is also known to contaminate cereal grain during harvesting. For example, a UK survey of seed drills showed that 21% of cereal grain samples were contaminated with catchweed bedstraw (Tonkin and Phillipson 1973). Catchweed bedstraw and false cleavers are also considered competitive weeds in field crops in Canada (Hall et al. 1998; Malik and Vanden Born 1988), where an infestation of 100 plants/m<sup>2</sup> can decrease canola (*Brassica napus* L.) yield by 18% (Malik and Vanden Born 1987a). In addition, continuous use of acetolactate synthase inhibitors in cereal crops in Alberta, Canada has led to the evolution of resistance to these herbicides in false cleavers (Hall et al. 1998). These studies suggest that if threehorn bedstraw has similar properties, it has the potential to become an even more problematic weed, should it spread to the rest of the grain growing areas of Australia.

Germination of catchweed bedstraw is inhibited by light (Froud-Williams 1985) and few seeds germinate from the soil surface (Boyd and Van Acker 2003; Taylor 1999), with greatest seedling emergence occurring with shallow burial up to 5 cm (Boyd and Van Acker 2003; Froud-Williams 1985). Light also inhibits germination of false cleavers, but dormancy induced by light can be overcome by potassium nitrate (KNO<sub>3</sub>) and to a lesser extent by gibberellic acid (GA<sub>3</sub>) (Malik and Vanden Born 1987b). Germination of false cleavers and catchweed bedstraw has been reported to be influenced by tillage (Reid and Van Acker 2005). However, such information on seed biology is not available for threehorn bedstraw.

Common sowthistle (*Sonchus oleraceus* L.) is another important dicotyledonous winter annual weed in southern Australia. It is a prolific seed-producing weed (Hutchinson et al. 1984), which can produce up to 8000 seeds per plant. It has become a serious problem in winter crops throughout southern Australia. This weed has evolved resistance to chlorsulfuron (acetolactate synthase inhibitor) in southern Queensland and northern New South Wales (Adkins et al. 1997). This weed has been recognised as a problem in conservation tillage systems in the northern cropping region of Australia (Widderick et al. 1999). Increasing abundance of this species in southern Australia could be related to the adoption of no-till (NT) systems as there is greater seedling emergence under reduced tillage systems than the conventional tillage systems (Widderick et al. 2004).

Wild turnip (*Brassica tournefortii* Gouan.) is an important dicotyledonous weed in southern Australia, which can cause a significant yield loss in crops even when present at a low density. One plant of wild turnip per square meter can cause 0.35% yield-loss in wheat (Gill and Davidson 2000). This weed has already evolved resistance to chlorsulfuron (inhibitor of acetolactate synthase) in Western Australia and South Australia (Heap 2006). Seed germination of a closely related species, canola, has been reported to be stimulated by light (Pekrun et al. 1997). In contrast, seed germination in wild radish (*Raphanus raphanistrum* L.), another Brassicaceae weed, is stimulated by darkness (Cheam and Code 1995).

Indian hedge mustard (*Sisymbrium orientale* L.), a small-seeded species, is a member of Brassicaceae family. It infests many crops and pastures in Australia (Amor and Fransisco 1987). The life cycle of Indian hedge mustard is closely associated with winter crops such as oilseed rape, field peas and chickpeas and it can compete severely with these crops (Amor and Fransisco 1987). It is an indeterminate plant and produces many flowers and seeds in the late winter and spring. Biotypes resistant to the acetolactate synthase inhibiting herbicides were reported in New South Wales, Queensland and South Australia (Adkins et al. 1997; Boutsalis and Powles 1995). A small percentage of the Indian hedge mustard seed bank can persist for longer than 5 years (Boutsalis and Powles 1998). Anecdotal evidence suggests that Indian hedge mustard may be increasing in abundance under NT conditions in southern Australia. Light and nitrate have been reported to stimulate germination in the seeds of a related species, hedge mustard [*S. officinale* (L.) Scop.] (Bouwmeester and Karssen 1993; Hilhorst 1990a, b). Seedling emergence of London rocket (*S. irio* L.) was optimal at a shallow seed burial depth of 2 mm (Ray et al. 2005).

Small-flowered mallow (*Malva parviflora* L.), an important dicotyledonous weed, is increasingly becoming a problem in the Australian farming systems. It is a weed of wasteland, crops and pastures throughout Australia (Michael et al. 2004). Small-flowered mallow is a self-pollinated species, which enables even a single plant to colonise a new habitat. This weed can be difficult to control with herbicides and changing farming practices, such as NT that rely heavily on glyphosate, have facilitated its increase as a weed problem. Seeds of this weed species usually require scarification to germinate (Chorbadjian and Kogan 2004; Muniz 2000). There is little information on the seed germination biology of small-flowered mallow in Australia.

A closely related species, round-leaved mallow (*M. pusilla* Sm.) has been well studied. In a review Makowski and Morrison (1989) concluded that round-leaved mallow is frequently found in cultivated fields, orchards, gardens, farmyards near manure piles, along roadsides, in towns and in waste places. They also described this species as salt tolerant and favouring nutrient-rich soils. In the absence of scarification, seeds of round-leaved mallow exhibit low germination and can persist in the soil for a long time. Weed surveys indicate that round-leaved mallow doubled in abundance on croplands in Alberta, Canada from 1980 to 1985. Round-leaved mallow is not only a prolific seed producer, but can also cause yield losses of up to 30% in wheat (Makowski and Morrison 1989). Round-leaved mallow germination and emergence occur at soil temperatures ranging from 5 to 30 C but are optimal at 15 to 20 C (Blackshaw 1990). Seedling emergence of round-leaved mallow was greatest at shallow depths of 0.5 to 2 cm (Blackshaw 1990). Such studies indicate that small-flowered mallow may also have the potential to become a more problematic weed in Australia.

Annual ryegrass (Lolium rigidum Gaud.) is a significant weed of wheat-based cropping systems in southern Australia (Gill 1996) and has now evolved resistance to many herbicides with different modes of action (Heap 2004; Preston 2000). The evolution of herbicide resistance means other strategies need to be developed for the management of this species. Understanding the germination and seedling emergence patterns of this species is critical and valuable in developing future management strategies. Primary seed dormancy is the main cause for the survival of this weed in the field (Steadman et al. 2003b), reducing germination in response to sporadic summer rainfall events, but allowing germination during the subsequent autumn and winter when the plant has a better chance of establishment and survival. Weed germination can differ among seeds collected in different years and from different mother plants (Andersson and Milberg 1998; Beckstead et al. 1996). However in annual ryegrass, it is unknown whether seeds produced from different populations collected from the same farm exhibit similar or variable germination and dormancy. There have been some studies on the dormancy of annual ryegrass; however, in those studies seeds were either after-ripened at a constant temperature (Steadman et al. 2003b, 2004b) or under a shelter in the field (Steadman et al. 2003a, 2004a).

Over the past decade, there has been a rapid increase in the use of reduced tillage for crop production in Australia. In 2001, more than 40% of the cropping area in Australia was under NT and over the next few years, this area is expected to increase dramatically (D'Emden and Llewellyn 2004). These changes in tillage practices may have a major influence on weed ecology. The soil disturbance caused by tillage systems places weed seeds at different depths, which differ in availability of moisture, diurnal temperature fluctuation, light exposure, and activity of predators. All these attributes of the microenvironment have the potential to influence seed germination. Consequently, changes in the dormancy or germination during burial of seeds may be expected (Gleichsner and Appleby 1989; Omami et al. 1999; Zorner et al. 1984). Burial of weed seeds to different depths can also affect seedling emergence from the seed bank (Benvenuti et al. 2001).

Weed seeds of different populations matured in different environments have been shown to have different levels of dormancy (Andersson and Milberg 1998; Milberg et al. 1996). In the field, there are wide ranges of factors that could influence dormancy release including temperature changes, wetting and drying cycles and predation. It is important that laboratory studies can be correlated with field observations.

Despite the growing concerns about weed shifts with changing tillage practices in southern Australian farming systems, knowledge of seed biology of key weeds is limited. In addition, the influence of various stresses such as salt and osmotic stress on seed germination of many of these weed species is also unknown. Soil salinity is a major constraint in Australia where more than 60% of the 20 million hectares of cropping soils are sodic and crops are generally grown on these soils under dryland conditions (Rengasamy 2002). These soil factors could also affect seed germination of these weed species.

The objectives of this study were to (1) determine the influence of various environmental factors on weed seed germination and seedling emergence, and (2) determine the fate of weed seeds when after-ripened at different depths in the field.

# **3.2 Materials and Methods**

## 3.2.1 Seed Description

Several experiments were conducted in 2004 through 2006 at the Roseworthy Campus of the University of Adelaide, South Australia. Seeds of six weed species (threehorn bedstraw, common sowthistle, wild turnip, Indian hedge mustard, smallflowered mallow and annual ryegrass) were collected at maturity in November through December of the year prior to the experiments. Seeds were considered mature when the weed plants had completely senesced. Seeds were threshed and separated from chaff manually.

Seeds of threehorn bedstraw were collected from several wheat fields at the Roseworthy Campus (RC) and in Warooka, on the Yorke Peninsula (YP) of South Australia. The distance between the two collection sites was 200 km. Seeds of common sowthistle and annual ryegrass were collected from wheat fields at the Roseworthy Campus. Wild turnip seeds were collected from wheat fields at Darke

Peak on the Eyre Peninsula of South Australia. Seeds of two populations of Indian hedge mustard, AFS and E5, were collected locally from wheat fields for the experiments conducted in 2005. The seeds of AFS population were used for all the experiments, unless otherwise specified. While seeds of only one population were collected for the experiments conducted in 2006. Seeds of small-flowered mallow were collected from the fence lines or roadsides at the Roseworthy Campus. Seeds of only one local population of annual ryegrass were collected for the 2004-05 experiments, while for the 2005 experiments seeds of five different populations (AFS, E1, E5, E9 and NW7) were collected from different fields on the same farm. Although these populations experienced similar climatic conditions, annual ryegrass is known to possess high genetic diversity (Gill 1996). Therefore, populations in different fields may have different genetic backgrounds, which may influence the behaviour of the seed bank.

Seeds of all species were stored dry in a glasshouse to maintain the light and temperature similar to the ambient conditions until the beginning of the experiments. The glasshouse temperature fluctuated between  $25 \pm 5$  C during the day and  $15 \pm 5$  C during the night. The seed size of different weed species is presented in Table 3.1.

Weed species	1000-seed weight		
	g		
Threehorn bedstraw	5.28-5.41*		
Common sowthistle	0.32		
Wild turnip	0.99		
Indian hedge mustard	0.04*		
Small-flowered mallow	3.46		
Annual ryegrass	2.43-2.93*		

 Table 3.1. Weed species and their seed weight.

\*Represents seed weight of different populations used in this study.

## **3.2.2 Germination Tests**

Seed germination was evaluated by evenly placing at least 25 seeds in a 9 cmdiameter Petri dish containing two layers of Whatman No. 1 filter paper, moistened with either 5 ml distilled water or a treatment solution, unless otherwise specified. Dishes were sealed with Parafilm<sup>1</sup> and placed in incubators at fluctuating day/night temperatures of 13/7 C (threehorn bedstraw), 20/12 C (common sowthistle, wild turnip and annual ryegrass) or 25/15 C (Indian hedge mustard and small-flowered mallow), unless otherwise specified. The photoperiod was set at 12 hours (h) to coincide with the high temperature period. Fluorescent lamps were used to produce a light intensity of 85  $\mu$ mol/m<sup>2</sup>/s. For germination in complete darkness, dishes were wrapped in two layers of aluminium foil. The number of germinated seeds was counted 14 days after the start of the experiment, with the criterion for germination being visible protrusion of the radicle. Seeds of small-flowered mallow were scarified with 95% sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) for 60 min before use (Chorbadjian and Kogan 2004). Treatments of each experiment were replicated three times.

Tetrazolium chloride (2,3,5-triphenyltetrazolium chloride) was used to test the viability of the ungerminated seeds of threehorn bedstraw and annual ryegrass (Steadman et al. 2003a). Non-germinated seeds of common sowthistle and turnip weed were subjected to a simple pressure test to determine whether the embryos were still viable (Taylor et al. 2004). Firm or hard seeds were considered as viable and soft seeds that lacked structural integrity were considered as non-viable or decayed. The viability of non-germinated seeds of small-flowered mallow was greater than 85% in all the experiments (data not shown). To check the viability in this species, the non-germinated seeds were given a cut with a scalpel and placed in the incubator as described above. Seed germination was counted after 7 days of incubation.

#### 3.2.3 Effect of Temperature, Light and Seed Age on Germination

The main objective of this experiment was to find the optimum germination temperature and light regime. Seed germination was determined in growth chambers under fluctuating day/night temperatures (25/15, 20/12, 13/7 or 15/9 C). These temperature regimes were selected to reflect the temperature variation during the

<sup>&</sup>lt;sup>1</sup>Parafilm, Pechiney Plastic Packaging, 289 River Street, Menasha, WI 54952.

autumn-winter period in southern Australia. Germination was determined under both light/dark and dark regimes. Each experiment was conducted twice.

Germination of dry after-ripened seeds (in the glasshouse) was also assessed at different seed age by placing seeds in the growth chambers as described above. In case of common sowthistle, germination was assessed by placing seeds in darkness for 28 days (dark), in a 12 h photoperiod for 28 days (light/dark) or in darkness for 14 days followed by a 12 h photoperiod for 14 days (dark followed by light/dark). Germination was counted 28 days after the start of the experiment. For small-flowered mallow, non-scarified seeds were used in this experiment.

To test the effects of after-ripening on threehorn bedstraw germination, further samples of both populations (RC and YP) were placed in the light/dark regime for 14 days at 3 and 6 months after seed maturity. The dishes were then placed in the dark for another 7 days. Germination was examined after this period and the number of germinated seeds counted. The experiment was conducted twice.

## 3.2.4 Effect of Scarification and Leaching on Germination

To test whether the inhibition of threehorn bedstraw germination was due to an impermeable seed coat, seeds of the RC population were physically scarified by cutting the seed coat with a scalpel. The scarified seeds were then incubated in the darkness at 13/7 C.

Two different experiments were conducted to test whether the inhibition of smallflowered mallow germination was due to an impermeable seed coat. In the first experiment, seeds were either physically scarified by cutting the seed coat with a scalpel or placed in boiling water for 5 minutes (min). There was also a control treatment (non-scarified seeds). In the second experiment, seeds were scarified with 95% H<sub>2</sub>SO<sub>4</sub> for different times (0, 10, 30, 60, 90, 120, 180, 240 and 300 min). Seeds were washed in running tap water for 5 min before placing in an incubator set at 25/15 C in a 12 h light/dark regime. The effect of leaching on seed germination of threehorn bedstraw was examined by placing seeds of both populations in permeable nylon bags that were then placed in water for imbibing and leaching. Water was changed whenever samples were taken for germination tests. Following various periods of leaching (0, 2, 4, 6, 24 and 48 h), samples of 25 seeds were removed and placed at 13/7 C in darkness for 14 days to test germination. Each experiment was conducted twice.

#### 3.2.5 Effect of Germination Media, KNO3 and GA3 on Germination

The effect of different germination media on seed germination of threehorn bedstraw was studied by incubating seeds in the dark. Seeds of the YP population were placed in Petri dishes containing distilled water, field soil, 0.01 M KNO<sub>3</sub><sup>2</sup>, 0.1 M KNO<sub>3</sub>, 0.001 M GA<sub>3</sub><sup>3</sup>, 2% soil extract (10 g soil in 500 ml distilled water) and 4% soil extract (20 g soil in 500 ml distilled water). The soil used in the experiment was a clay loam (8 mg/kg NO<sub>3</sub>-N, 0.15% total nitrogen, pH 7.5, 1.5% organic carbon). Soil extract was used as it has been reported to stimulate germination of false cleavers (Malik and Vanden Born 1987b). Where the soil medium was used, seeds were buried in the soil rather than being placed on the soil surface.

A preliminary experiment was conducted under the light/dark and dark conditions to study the effects of KNO<sub>3</sub> on germination of the RC population of threehorn bedstraw. When no germination was found in the light/dark regime, detailed experiments were performed by incubating seeds only in the darkness. Seeds of both populations (three months after maturity) were treated with different concentrations of KNO<sub>3</sub> (from 0 to 0.1 M) and in combination with 0.001 M GA<sub>3</sub>. Germination was analysed separately for each population and allowing for the interaction of KNO<sub>3</sub> and GA<sub>3</sub>. The effect of different concentrations of KNO<sub>3</sub> (0 to 0.08 M) and GA<sub>3</sub> (0.001 M) on seed germination of wild turnip, Indian hedge mustard and small-flowered mallow was also determined. Seed germination of wild turnip and Indian hedge mustard was conducted in both light/dark and dark conditions and small-flowered mallow in light/dark. Each experiment was conducted twice.

<sup>&</sup>lt;sup>2</sup>Potassium nitrate, VWR International Ltd., Merck House Poole, Dorset BH 15 1TD, England. <sup>3</sup>Cibberglia goid Sigma Aldrick Co. 2050 Sprace Street St. Lewis MO (2102)

<sup>&</sup>lt;sup>3</sup>Gibberellic acid, Sigma-Aldrich Co., 3050 Spruce Street, St. Louis, MO 63103.

#### 3.2.6 Effect of Seed Size on Germination of Threehorn Bedstraw

Seed size effects on germination of threehorn bedstraw were studied at 0, 3, 6 and 9 months after seed maturity in both light/dark and dark conditions. Cleaned seeds of the RC population were sieved through a 2-mm diameter aperture sieve. Seeds that passed through the sieve were categorised as small seeds and the others as large seeds. In a random sample, 30% of seeds were in the small seeds category and 70% in the large. The seeds of different sizes were tested for germination by placing on water-moistened filter paper in Petri dishes. Seeds were placed at 13/7 C in the dark or in an alternating light/dark regime for 14 days.

#### 3.2.7 Effect of Cold Stratification on Germination

Seeds of the RC population of threehorn bedstraw that had been stored on the soil surface for 5 months after maturity in a field were subjected to cold stratification at 5 C for 5 weeks. Fifty seeds were placed in Petri dishes containing moist sand in continuous darkness at 5 C. Every week, the dishes were removed from the incubator and seeds exhumed from the dishes. Seeds that had germinated at 5 C were counted and represented the germination that occurred at 5 C. The ungerminated seeds were placed on filter paper and incubated at 13/7 C in both light/dark and dark regimes. Seeds germinating at 13/7 C were counted after 14 days of incubation at this temperature regime and calculated as a percentage of the ungerminated seeds.

In addition, the effects of temperature, time (week of incubation) and  $KNO_3$  (0.005 M) on germination of RC population of threehorn bedstraw were tested. Seeds were incubated at 5 C (cold stratification temperature) or 13/7 C (optimum temperature). Four sets of dishes were placed in each incubator. Every week, one set was removed from the incubators and germination was checked.

Non-scarified seeds of small-flowered mallow were placed in Petri dishes containing sand and chilled at 5 C for 0, 10, 20, 30, and 40 days in continuous darkness. For each treatment, the dishes were removed from the incubator, seeds were exhumed from the sand, placed on filter papers and incubated at 25/15 C in the light/dark

regime. Seed germination was counted 14 days after exhumation. Each experiment was conducted twice.

#### 3.2.8 Effect of Salt and Osmotic Stress on Germination

Weed seed germination as affected by salt stress was studied by using sodium chloride  $(NaCl)^4$  solutions of 0, 10, 20, 40, 80, 160, and 320 mM. For osmotic stress, water solutions with osmotic potentials of 0, -0.1, -0.2, -0.4, -0.6, -0.8 and -1.0 MPa were prepared by dissolving known amounts of polyethylene glycol<sup>5</sup> 8000 in 1 L of distilled water according to Michel (1983). These ranges of NaCl and osmotic potentials were selected to reflect the level of salinity and water stress occurring in southern Australian soils. In these experiments, seeds of YP population of threehorn bedstraw and E5 population of annual ryegrass were used. The effect of stresses on seed germination of Indian hedge mustard was studied in both 2005 and 2006. Each experiment was conducted twice.

#### **3.2.9 Effect of Age and Depth on the Fate of Seed**

Samples of 25 seeds of threehorn bedstraw (both populations, RC and YP), common sowthistle and wild turnip were placed in 7 cm by 7 cm permeable nylon bags (1.0 mm by 0.5 mm pore size) separately. Permeable bags were used to create natural soil conditions (water and air diffusion, and microorganisms). Initially, the bags were placed on the field soil surface on 15 January, 2005. At 1.5 and 3 months of afterripening on the soil surface, the bags were sampled to assess germination. After 3 months (in mid April), two-thirds of the bags were buried at 2 and 5 cm to simulate tillage and other one-third remained on the soil surface. In southern Australia, farmers practicing conventional tillage usually cultivate their land around this time of the year (April to May) after the initial rainfall events in late autumn. These burial depths were selected to reflect the vertical seed distribution by the commonly occurring tillage systems in southern Australia. The texture of the field soil was clay loam with 1.5% organic carbon and a pH of 7.5.

<sup>&</sup>lt;sup>4</sup>Sodium chloride, Asia Pacific Specialty Chemicals Ltd., ABN 32000316138.

<sup>&</sup>lt;sup>5</sup>Polyethylene glycol 8000, Sigma-Aldrich Co., 3050 Spruce Street, St. Louis, MO 63103.

The bags were arranged in a randomised complete block design with three replicates. The bags containing seeds were exhumed from the field at an interval of 1.5 months and the ungerminated seeds were incubated in the light/dark (common sowthistle) and dark (threehorn bedstraw and wild turnip) conditions for 14 days. After burial of seeds, comparisons between seed burial depth over time (months after seed burial) were made for different components of seed fate (germination in the field and laboratory, decayed and dormant seed). For threehorn bedstraw, only laboratory germination was recorded.

Various experiments were conducted in 2004-05 and 2005 with the seeds of annual ryegrass. In 2005, a study was undertaken to determine if there were differences in the various components of seed fate (field and laboratory germination, decayed and dormant seed) between seeds of five different populations (AFS, E1, E5, E9 and NW7) collected from different fields on the same farm. Samples of 40 seeds of each population were placed in nylon bags. After harvest of the previous crop, the bags were placed on the soil surface in the field on 15 January, 2005. The bags containing seeds were taken from the field at 1.5 months intervals for 10.5 months. The germination tests were conducted by placing dishes at 20/12 C in light/dark.

In the experiment conducted in 2004-05, 30 seeds of annual ryegrass were placed with about 10 g non-sterile soil (to maintain natural microflora) in bags. The soil was sieved through a 0.25-mm diameter sieve prior to mixing with seeds to avoid inclusion of other annual ryegrass seeds. After harvest of the previous crop, all bags were placed in a field on 12 January, 2004. The field soil was heavy clay with an organic carbon content of 2.0%, and a pH of 7.8. The bags were placed on the soil surface with or without residue or buried at 5 cm without residue. In the residue treatment, wheat residue (3 t/ha) was applied to cover the seed bags. This rate of residue is characteristic of that usually occurring in NT farming systems in the area. The experimental area was covered with nylon net to prevent damage to seeds by birds and to prevent the residue from being blown away by wind. The bags containing seeds were exhumed from the field at 1, 2, 3, 4, 5, 7, 10 and 16 months after seed storage. The seeds were washed to remove soil before undertaking germination tests as described above.

In the experiment conducted in 2005, samples of 25 seeds of annual ryegrass of the E5 population were placed in bags. The bags were placed in a field on the soil surface on 01 January, 2005. After 4 months in the field, two-thirds of the bags were buried at 2 and 5 cm to simulate the effects of tillage. The other one-third of the bags remained on the soil surface. The bags were exhumed from the field at monthly intervals from 2 to 12 months and the seeds incubated at 20/12 C in the light/dark conditions for 14 days.

Seed germination in the field was evaluated by counting the established plants at the time of sampling and non-viable seeds were considered as decayed seeds. Fatal germination (non-established plants) would have been included under the seed decay category, as these seeds did not establish into seedlings. Therefore, the term 'seed decay' has been used to describe losses due to natural decay and fatal germination.

In this experiment, germination of threehorn bedstraw and annual ryegrass was evaluated by evenly placing seeds in a 9 cm-diameter Petri dish containing 1% agar. For other species, seeds were placed on the filter papers. The daily maximum and minimum temperature in the field was recorded in 2005 using Tinytag temperature data loggers<sup>6</sup> in all the seed storage environments. The average monthly maximum and minimum temperatures are presented in Table 3.2.

#### **3.2.10 Effect of Seed Burial Depth on Seedling Emergence**

The effect of seed burial depth on seedling emergence was studied in fields for threehorn bedstraw (RC and YP populations) and annual ryegrass (E5 population) or in growth chambers for common sowthistle, wild turnip and Indian hedge mustard or in a glasshouse for small-flowered mallow. The temperature of the growth chambers was set at 20/12 C for common sowthistle and wild turnip and 25/15 C for Indian hedge mustard. The temperature of the glasshouse fluctuated between  $25/15 \pm 5 \text{ C}$  (day/night temperature). Fifty seeds of each weed species were placed on the soil surface and then covered with soil to achieve different burial depths. Pots (15-cm diameter) were used for common sowthistle, wild turnip and small-flowered mallow,

<sup>&</sup>lt;sup>6</sup>Tinytag temperature data loggers, Hastings Data Loggers, New South Wales 2444, Australia.

while 15-cm diameter Petri dishes were used for Indian hedge mustard. The seedling emergence of annual ryegrass was studied in 2004 and 2005, while Indian hedge mustard was studied in 2005 and 2006. The seedling emergence of other species was studied only in 2005. Control area/pots/dishes, where seeds were not added, indicated that there was no background seed bank of these weed species in the study soil. Seedlings were counted as they emerged through the soil. The experiment was terminated when no further emergence was recorded on three consecutive measurements. Each experiment was conducted twice.

Months	Maximum temperature			Minimum temperature					
-	0 cm	2 cm	5 cm	0 cm	2 cm	5 cm			
	C								
January	44.9	43.0	35.9	17.7	18.2	20.5			
February	40.5	39.2	32.6	15.5	15.5	18.1			
March	39.6	37.1	31.4	13.9	13.9	16.7			
April	33.1	33.6	28.0	13.8	13.6	16.0			
May	26.2	26.6	21.8	9.0	9.2	11.8			
June	17.7	18.6	16.0	7.8	7.4	9.0			
July	15.7	17.3	14.7	7.0	6.4	7.7			
August	17.0	19.8	15.6	7.5	6.8	8.4			
September	18.9	18.5	18.6	8.4	9.3	10.6			
October	22.6	19.7	22.4	9.3	12.0	13.2			
November	31.2	32.7	30.2	10.1	14.9	16.6			
December	34.7	36.9	34.1	12.4	17.9	19.3			

**Table 3.2.** Average monthly maximum and minimum temperatures recorded in 2005at burial depth of 0, 2 and 5 cm.

## 3.2.11 Effect of Tillage Systems on Seedling Emergence Patterns

This experiment was conducted during the growing season of 2005 on the Roseworthy Campus Farm of the University of Adelaide, South Australia. The tillage systems comprised of NT and minimum-tillage (MINT). Seeds of two populations (RC and YP) of threehorn bedstraw (200 seeds/m<sup>2</sup>) and two populations (AFS and E5) of Indian hedge mustard (500 seeds/m<sup>2</sup>) were spread in fixed quadrats (size 2 m

by 1 m) in mid March of 2005. Control plots, where seeds were not spread, indicated that there was no background seed bank of either species in the study area.

Two cultivations were given to the MINT plots to a depth of 8 cm before crop sowing. The width of the tines used for cultivation was 100-mm. In the NT plots, soil disturbance was limited to the sowing operation only. Wheat cv. Krichauff was sown with a seeder fitted with knife-point soil openers (16-mm wide) in the NT plots, while 100-mm wide tines (to create more soil disturbance) were used for sowing in the MINT plots. The crop was sown in rows 25-cm apart on 17 June, 2005. Weed seedling emergence was measured at different times after crop sowing (days after sowing, DAS) from the whole plot and expressed as a percentage of the seed bank at sowing. The census of seedlings was discontinued when no further emergence was recorded on three consecutive measurements.

#### **3.2.12 Statistical Analyses**

All experiments except the tillage experiment were conducted in a randomised complete block design. Treatments of each experiment were replicated three times. For the seed fate experiment, data before and after seed burial were analysed separately. After seed burial, separate analyses were performed on the basis of total seeds for: (a) germination in the field (only established seedlings); (b) seed decay; (c) dormant seeds (firm/hard seeds); and (d) germination in the laboratory.

The tillage experiment was arranged in a split-plot design with tillage systems as the main-plots and populations as the sub-plots. There were three replicates of each treatment. Seedling emergence of each population under MINT and NT systems was analysed at each sampling time by using analysis of variance (ANOVA). Seedling emergence values for each population under MINT and NT were fitted to functional three-parameter sigmoid and logistic models.

In all cases, experiment run was not significant and because there were no significant run by treatment interactions data were pooled over runs and re-analysed. Percent germination data for all experiments were transformed. Data variance was visually inspected by plotting residuals to confirm homogeneity of variance before statistical analysis. Transformation of data did not improve homogeneity of variance; thus, ANOVA was performed on nontransformed percent germination. In some experiments, regression analysis was more appropriate for the data. In other experiments, means were separated using LSD (P = 0.05). Genstat version 6.0 was used for statistical analyses (Genstat 5 Committee 1993).

## **3.3 Results and Discussion**

#### 3.3.1 Effects of Temperature, Light and Seed Age on Germination

#### 3.3.1.1 Threehorn Bedstraw

Seeds from the RC and YP populations of threehorn bedstraw were subjected to three temperature ranges (25/15, 20/12 and 13/7 C) and two light regimes (light/dark and dark). Germination at the time of seed maturity and 11 months after maturity was achieved only at the alternating temperature regime of 13/7 C and in complete darkness. No germination was observed under any other temperature regime or in the light. Germination at the time of seed maturity was low for both populations (15.6 to 17.8%); however, the viability of the seeds as determined by the tetrazolium chloride test was almost 100% for both populations (data not shown). This suggests that the inhibition of germination immediately after maturity was very high in these populations. Even though germination 11 months after seed maturity increased for both populations (51.7 to 55.0%), a considerable proportion of seeds still did not germinate. In addition, light inhibited germination even at 13/7 C.

The results suggest that threehorn bedstraw can germinate only at low temperatures, which would usually occur from June to August in southern Australia. The optimum temperature ranges reported for germination of related species are variable and generally lower for catchweed bedstraw than false cleavers. Similar to this study, lower optimum temperature ranges of 9 to 12 C (Froud-Williams 1985) and 12 to 15 C (Sjostedt 1959) have been reported for catchweed bedstraw. The results also demonstrate that light inhibited germination in this species which is similar to the response of false cleavers to light (Malik and Vanden Born 1987b). The results suggest that threehorn bedstraw may be similar to other negative photoblastic seeds in which phytochrome far-red ( $P_{\rm fr}$ ) remaining after seed ripening may trigger

germination upon rehydration in the dark (Rollin 1972). In such seeds, exposure to light during germination may convert the  $P_{\rm fr}$  to the inactive phytochrome red ( $P_{\rm r}$ ) form.

Reversibility of light-inhibited germination by subsequent dark incubation was studied with seeds of both populations at 3 and 6 months after seed maturity. Germination was not observed during the initial light/dark incubation for 14 days. However, subsequent dark incubation for another 7 days enabled germination in both populations (Table 3.3). The germination percentage after this treatment was similar among populations and times after maturity at 53 and 55% and 60 and 65% for the RC and YP populations at 3 and 6 months after seed maturity, respectively. Light-inhibited germination in seeds of threehorn bedstraw can be alleviated by a period in the dark. The requirement of darkness for germination in this species means that a high proportion of seeds will remain ungerminated on the soil surface, either until seed death (due to desiccation or predation activity) or until burial occurs through tillage or sowing operations. Therefore, greater seedling emergence would be expected in tilled fields compared to untilled fields.

**Table 3.3.** Effects of subsequent dark incubation of light-inhibited seeds on germination of RC and YP population of threehorn bedstraw at 3 and 6 months after seed maturity.

Time	Germination						
	RC			YP			
	Light/dark	Dark		Light/dark	Dark		
month			%				
3	0	53		0	60		
6	0	55		0	65		
LSD 0.05	14			——16——			

Germination of dry after-ripened seeds of threehorn bedstraw in the glasshouse was influenced only by seed age. Regardless of the population, around 50% germination was achieved after 3 months of storage and this level did not change with seed age (Figure 3.1).



**Figure 3.1.** Seed germination of RC and YP populations of threehorn bedstraw when after-ripened dry in a glasshouse. Germination was tested by incubating seeds at 13/7 C (12 h/12 h) in darkness for 14 days.

#### 3.3.1.2 Common Sowthistle

Germination of freshly harvested common sowthistle seeds was not affected by temperature (25/15, 20/12 and 15/9 C); however, germination was stimulated by the presence of light. Seed germination at 25/15, 20/12 and 15/9 C was 80, 93 and 84% under light/dark conditions and only 44, 47 and 40% in darkness. There appears to be a low level of dormancy in common sowthistle with greater than 90% germination at seed maturity.

The ability to germinate over a broad range of temperatures at the time of maturity is probably one of the reasons why common sowthistle emerges throughout the growing season in southern Australia (e.g. Widderick et al. 2004). Stimulation of common sowthistle germination by light is consistent with previous studies (Andersson et al. 1997; Widderick et al. 2004). Andersson et al. (1997) found 100% germination in light, whereas 75% of seeds germinated in darkness. Widderick et al. (2004) reported only 20% germination in darkness, which was lower than that reported in our study. Greater germination of common sowthistle seeds in light (under optimum conditions) suggests that its germination and subsequent emergence in the field will be favoured by the presence of seeds at or near the soil surface. Such an environment is created under NT systems where a large proportion of the weed seed bank will be on or close to the soil surface.

Seeds of common sowthistle after-ripened in the glasshouse always showed greater germination under light/dark conditions compared to dark (Figure 3.2). Dark incubation for 14 days followed by exposure to light (light/dark) for 14 days resulted in equal germination to seeds incubated in light/dark for the entire duration. This indicates that incubation in the dark does not induce secondary dormancy in the seeds of this species. The proportion of seed that does not require light for germination is likely to germinate when seeds are buried, provided moisture and temperature are suitable. However, germination of seeds buried deep in soil is likely to prove fatal for this small-seeded species. Between 12 to 40% of seeds germinated readily in the darkness while the rest of the seeds required light for germination.



Figure 3.2. Effect of afterripened seeds (in а glasshouse) on germination common of sowthistle. Germination was tested by placing seeds at 20/12 C in darkness for 28 days (dark), with a 12 h photoperiod for 28 days (light/dark), or in 14 darkness for days followed by a 12 h photoperiod for 14 days (dark fb light/dark).

#### 3.3.1.3 Wild Turnip

Seed germination of wild turnip, at the time of maturity, was influenced by the interaction between temperature and light (Figure 3.3). At higher temperatures (25/15 and 20/12 C), seed germination was unaffected (P > 0.05) by the light conditions. While at the lowest temperature (15/9 C), germination was significantly lower in light/dark (7%) than in the dark (62%). The viability of the seeds as determined by the pressure test was greater than 90% (data not shown).



**Figure 3.3.** Effect of temperature and light on germination of wild turnip seeds at maturity. Vertical bars with caps represent standard errors.

The results indicate that germination of wild turnip seeds is inhibited by light at suboptimal temperatures. Cousens et al. (1993) had also reported that seeds of wild turnip had a distinct preference for darkness when incubated at lower temperature (10 C) compared to the higher temperature (> 15 C). Similarly, Thanos et al. (1991) also reported inhibition of germination for wild turnip in continuous darkness. In contrast, seed germination in canola (a related species) was stimulated by exposure to light at 12 or 20 C (Pekrun et al. 1997). The results suggest that wild turnip may be similar to other negative photoblastic seeds in which P<sub>fr</sub> remaining after seed ripening may trigger germination upon rehydration in the dark (Rollin 1972).

The effect of a dry after-ripening period on the germinability of wild turnip seeds was investigated at 20/12 C in the light/dark and dark regimes at 0, 1.5, 3, 4.5, 6, 9, 10.5 and 12 months after seed maturity. Germination was not influenced by the light conditions (Figure 3.4), which is supported by the result of the temperature and light experiment where germination at 20/12 C was similar between light/dark and dark conditions. However, it appears that there was decline in seed germination during the colder months of the year.

#### 3.3.1.4 Indian Hedge Mustard

Seed germination of Indian hedge mustard (at 25/15 C day/night temperature) was stimulated by light (Figure 3.5). Regardless of the light condition, germination was low at seed maturity (< 20%). With light/dark incubation, the germination percentage

increased with time from maturity reaching 61% by 12 months after maturity. While in the dark incubation, germination never exceeded 21%.



**Figure 3.4.** Germination pattern of dry after-ripened seeds (in a glasshouse) of wild turnip. Germination was tested by placing seeds at 20/12 C in the light/dark and dark conditions. Vertical bars represent standard errors.

**Figure 3.5.** Germination of dry after-ripened seeds (in a glasshouse) of Indian hedge mustard. Germination was tested by placing seeds at 25/15 C in light/dark and dark conditions.

Similar to this study, Boutsalis and Powles (1998) reported a requirement for an after-ripening period in Indian hedge mustard. Stimulation of germination by light (optimum condition) indicates that seeds of Indian hedge mustard are positively photoblastic. Many small-seeded broadleaf weed species exhibit positive photoblasticity (e.g. Widderick et al. 2004), although others, such as hairy nightshade (*Solanum sarrachoides* Sendtner), have been reported to germinate equally well in light and dark (Zhou et al. 2005). Greater germination of Indian hedge mustard seeds in the light also suggests that its germination in the field will be favoured by presence

of seeds at the soil surface. Such an environment is created under NT systems where a large proportion of the weed seed bank will be on or close to the soil surface. There were up to 21% seeds, which germinated readily in the darkness. Therefore, some of the seeds that do not require light for germination are likely to germinate even when buried, provided moisture and temperature are suitable.

## 3.3.1.5 Small-Flowered Mallow

Germination of small-flowered mallow seeds without scarification was low (about 5%) at harvest (Figure 3.6). The germination percentage increased slowly with time from harvest reaching almost 50% by 13 months after harvest. This species clearly has an extended dormancy mechanism or mechanisms that inhibited germination in the first few months after harvest. Even then, dormancy was still evident after more than a year of storage. Round-leaved mallow has a similar pattern of release from dormancy (Makowski and Morrison 1989). The germination percentage of small-flowered mallow seeds in the dark was not different to that under a light/dark regime at any of the times tested (Figure 3.6).



**Figure 3.6.** Effect of seed age on germination of smallflowered mallow after 14 days of incubation in either light/dark or dark at 25/15 C in a 12 h photoperiod.

#### 3.3.2 Effect of Scarification and Leaching on Germination

In threehorn bedstraw, germination caused by scarification (cutting) was similar to germination of intact seeds (data not shown). This suggests that inhibition of germination in threehorn bedstraw is not due to an impermeable seed coat.
Inhibition of germination may be the result of germination inhibitors present in the seed or seed coat that need to be leached by rainfall before the seed will germinate. Germination of the RC and YP populations in the absence of leaching was 17 and 27%, respectively. However, leaching of seeds with water from 2 to 48 h inhibited germination (data not shown). This indicates that inhibition of germination in this species is unlikely to be the result of the presence of leachable germination inhibitors.

Germination of small-flowered mallow seeds was stimulated by scarification of the seed coat. In the first experiment, germination of non-scarified seeds (control), seeds placed in boiling water and physically scarified seeds (with a scalpel) was  $10.0 \pm 0$ ,  $20.8 \pm 1.7$  and  $87.5 \pm 1.4\%$ , respectively. Seed scarification by the scalpel was the most effective method for stimulation in germination; however, it is tedious and time consuming. This increase in germination due to scarification could be due to increased imbibition, the movement of inhibitors from within the embryo or the release of physical restriction by the seed coat. These results suggest that the inhibition of germination in this species was due in large part to an impermeable seed coat. Seeds of round-leaved mallow were also found to be impermeable to water, exhibiting low germination unless scarified (Makowski and Morrison 1989).

In order to find a faster method of scarification, seeds of small-flowered mallow were scarified with 95%  $H_2SO_4$  for different times (Chorbadjian and Kogan 2004). Seed germination increased with the time of  $H_2SO_4$  scarification up to 60 min and decreased after that (Figure 3.7). Seeds scarified for 60 min resulted in 59% germination compared with 12% for unscarified seeds. Therefore, for future experiments seeds were scarified with 95%  $H_2SO_4$  for 60 min, as this method was less time consuming than scarification with a scalpel.

The results showed that seeds of small-flowered mallow are unlikely to germinate in the field unless scarified. In the field, natural scarification is usually brought about by means such as trampling by animals, incomplete predation by insects or rodents, damage by fungi or soil microorganisms, passage through the digestive tract of animals, tillage implements and extreme changes in temperatures (e.g. Taylor 2005).

Under NT systems of cropping, scarification due to tillage would only occur at sowing. Consequently, small-flowered mallow would only emerge after crops have been sown thus making it more difficult to control. Alternatively, seeds sitting on the soil surface are more likely to be exposed to extreme temperature changes and may lose dormancy faster due to formation of cracks in the seed coat.



**Figure 3.7.** Effect of time of chemical scarification with 95% H<sub>2</sub>SO<sub>4</sub> on seed germination of small-flowered mallow incubated at 25/15 C in a 12 h photoperiod for 14 days.

## 3.3.3 Effect of Germination Media, KNO3 and GA3 on Germination

#### 3.3.3.1 Threehorn Bedstraw

Threehorn bedstraw germination was stimulated by 0.01 M KNO<sub>3</sub> while 0.1 M KNO<sub>3</sub> inhibited germination (Figure 3.8). Burying seeds in soil, or treating them with GA<sub>3</sub> also stimulated germination to the same level as 0.01 M KNO<sub>3</sub>. Soil and soil extracts have been reported to be better media than distilled water for germination of catchweed bedstraw and false cleavers (Malik and Vanden Born 1987b; Sjostedt 1959). Soil extracts are likely to contain soluble nitrates and this may be the reason for their ability to stimulate germination. However, germination of threehorn bedstraw was not stimulated by soil extracts. This could be the result of very low nitrate (8 mg/kg soil) present in the soil used to develop the extracts. There may also be other factors involved, as placing seed in the soil caused a stimulation of germination. Since the seeds tested have a relatively large size, it is possible that the 'soil effect' could be due to the better seed imbibition. In addition, ethylene (an important hormone) produced in the soil could have stimulated seed germination.



**Figure 3.8.** Effect of media on seed germination of the YP population of threehorn bedstraw when incubated at 13/7 C in darkness for 14 days. Vertical bars represent standard errors.

In a preliminary experiment, stimulation of germination in threehorn bedstraw by  $KNO_3$  only occurred in the darkness (data not shown). Therefore, experiments on the effects of  $KNO_3$  and  $GA_3$  were conducted only in the darkness (at 13/7 C). Germination of seeds of both populations (RC and YP) was stimulated by either  $KNO_3$  or  $GA_3$  (Figure 3.9; Table 3.4).



Figure 3.9. Effect of different concentrations of KNO<sub>3</sub>, with (open symbol) and without (solid symbol) GA<sub>3</sub>, on germination of the RC (circle) and YP (inverted triangle) populations of threehorn bedstraw at 13/7 C dark. Vertical in bars represent standard errors.

 $KNO_3$  at 0.005 M stimulated germination of both populations to a maximum level of 56% and 68%, respectively (Figure 3.9). However, germination of both populations started to decline with concentrations of  $KNO_3$  greater than 0.005 M. At the highest concentration of  $KNO_3$  (0.1 M), germination was lower than without  $KNO_3$ .

Germination of both populations was promoted by the combination of GA<sub>3</sub> with KNO<sub>3</sub>. However, the interaction between KNO<sub>3</sub> and GA<sub>3</sub> was found to be significant only in the YP population (Table 3.4). The difference in germination between GA<sub>3</sub> alone and combinations of KNO<sub>3</sub> with GA<sub>3</sub> was not large. While GA<sub>3</sub> can stimulate germination with KNO<sub>3</sub>, germination was still inhibited by greater concentrations of KNO<sub>3</sub> even when GA<sub>3</sub> was present. Germination promotion by KNO<sub>3</sub> has also been reported for false cleavers (Malik and Vanden Born 1987b).

**Table 3.4.** Degrees of freedom (df) and level of significance for the effects of  $KNO_3$  and  $GA_3$  and their interaction on germination of seeds of the RC and YP populations of threehorn bedstraw.

Factor	df	F probability		
	-	RC	YP	
KNO <sub>3</sub>	7	<i>P</i> < 0.001	<i>P</i> < 0.001	
GA <sub>3</sub>	1	P < 0.001	P < 0.001	
KNO <sub>3</sub> by GA <sub>3</sub>	7	NS	P < 0.01	

Residual mean square of RC population = 82.72 with 30 df.

Residual mean square of YP population = 39.62 with 30 df.

## 3.3.3.2 Wild Turnip

The effect of KNO<sub>3</sub> and GA<sub>3</sub> on germination of wild turnip seeds (at 20/12 C) was studied under both light/dark and dark regimes. However, the data was pooled over the light regimes, as the effect of light itself and its interaction with other factors were non-significant. Seed germination was significantly influenced by the interaction between KNO<sub>3</sub> and its combination with GA<sub>3</sub>. Germination was stimulated (low but significantly) by KNO<sub>3</sub> at low concentrations and its combination with GA<sub>3</sub> (Figure 3.10). KNO<sub>3</sub> at 0.005 M stimulated germination to a maximum level of 46% compared to 0 M KNO<sub>3</sub> (31%). However, germination started to decline with concentrations of KNO<sub>3</sub> greater than 0.005 M. At the greatest concentration of KNO<sub>3</sub> (0.08 M), germination was lower than without KNO<sub>3</sub>. Germination with GA<sub>3</sub> alone was similar to the combination of KNO<sub>3</sub> (from 0.005 to 0.04 M) and GA<sub>3</sub>.



Figure 3.10. Interaction effect of KNO3 and its combination with GA<sub>3</sub> on germination of wild turnip seeds when incubated at 20/12 C. itself Light and its interaction with KNO<sub>3</sub> and/or GA<sub>3</sub> was non significant, so data were pooled over the light conditions.

### 3.3.3.3 Indian Hedge Mustard

The effect of KNO<sub>3</sub> and GA<sub>3</sub> on germination of 1 month old Indian hedge mustard seeds was studied under both light/dark and dark regimes. Seed germination was significantly influenced (P < 0.001) by the interaction between light, KNO<sub>3</sub> and its combination with GA<sub>3</sub> (Table 3.5). Overall, germination was greater in light/dark than in dark (Figure 3.11). Germination was stimulated by KNO<sub>3</sub> at low concentrations and its combination with GA<sub>3</sub>. In light/dark, KNO<sub>3</sub> at 0.02 M stimulated germination to a maximum level of 45% compared to 0 M KNO<sub>3</sub> (18%). However, germination declined with concentrations of KNO<sub>3</sub> greater than 0.02 M. In dark, germination showed a small increase from the addition of GA<sub>3</sub> increased seed germination to 93% in this experiment, whereas seed germination in the seed age experiment was 61% in water even after 12 months of maturity. This response is likely to be due to presence of physiological dormancy in this species.

The results from this experiment suggest that field application of N-containing fertilisers may cause some stimulation of the germination of Indian hedge mustard seeds. Bouwmeester and Karssen (1993) also reported stimulation of germination in hedge mustard seeds by the addition of nitrate. However, the influence of nitrate on

germination is more likely for the seeds present on the soil surface (light) than those buried in the soil (dark).

Source of variation	df	SS	F probability
Light	1	11690	P < 0.001
KNO <sub>3</sub>	4	2070	P < 0.001
GA <sub>3</sub>	1	32550	P < 0.001
Light by KNO <sub>3</sub>	4	382	P < 0.001
Light by GA <sub>3</sub>	1	1576	P < 0.001
KNO <sub>3</sub> by GA <sub>3</sub>	4	2679	P < 0.001
Light by KNO <sub>3</sub> by GA <sub>3</sub>	4	684	P < 0.001
Error	38	546	

**Table 3.5.** ANOVA results for the effects of light,  $KNO_3$  and  $GA_3$  and their interactions on germination of Indian hedge mustard seeds in 2005<sup>a</sup>.

<sup>a</sup>Abbreviation: df, degree of freedom; SS, sum of squares.



**Figure 3.11.** Effect of different concentrations of  $KNO_3$  with and without  $GA_3$  on germination of Indian hedge mustard seeds when incubated at 25/15 C in light/dark and dark conditions.

#### 3.3.3.4 Small-Flowered Mallow

Seed germination of scarified seeds of small-flowered mallow was slightly increased by both KNO<sub>3</sub> and GA<sub>3</sub> (Figure 3.12). KNO<sub>3</sub> at 0.005 M stimulated seed germination to a maximum level of 76%. However, concentrations of KNO<sub>3</sub> greater than 0.01 M reduced germination. At the greatest concentration of KNO<sub>3</sub> (0.08 M), germination was only 3%. Seed germination of small-flowered mallow was also promoted by GA<sub>3</sub> (0.001 M) reaching 71%. Although the levels of nitrate in cropping soils are usually lower than used here, field application of N-containing fertilisers may slightly increase the germination of small-flowered mallow seeds. The results also indicate that in addition to the hard seed coat, embryo-based factors also contribute to seed dormancy in this species.



**Figure 3.12.** Effect of KNO<sub>3</sub> concentrations and 0.001 M GA<sub>3</sub> on germination of scarified seeds of small-flowered mallow when incubated at 25/15 C for 14 days.

KNO<sub>3</sub> and GA<sub>3</sub> are known to be agents that can overcome dormancy (Fawcett and Slife 1978; Hendricks and Taylorson 1974). Stimulation of seed germination by GA<sub>3</sub> has been shown in many plant species and could be due to mobilisation of food reserves in the seeds. The results from these experiments suggest that field application of N-containing fertilisers may have some influence on the germination of threehorn bedstraw, Indian hedge mustard, wild turnip and small-flowered mallow.

#### 3.3.4 Effect of Seed Size on Germination of Threehorn Bedstraw

Seed size of threehorn bedstraw had no impact on germination in the light/dark regime, as seeds of threehorn bedstraw did not germinate under these conditions. Therefore, data are shown only for the dark treatment (Figure 3.13). Germination was also not influenced by the size of the seeds in the dark; however, overall germination was greater at the later stages of the experiment than at the time of seed maturity. This observation indicates that germination in this species will increase

with time. In addition, small and large seeds had similar seed viability over the period of this study and will contribute to plant populations in the future.



Figure 3.13. Effect of seed sizes on germination of threehorn bedstraw (RC population) when tested in the darkness (at 13/7 C) at different times after seed maturity. Seeds were classified as small, < 2 mm (solid circle), and large, > 2 mm (open circle). Vertical bars represent standard errors.

# 3.3.5 Effect of Cold Stratification on Germination

# 3.3.5.1 Threehorn Bedstraw

Seeds of the RC population of threehorn bedstraw were chilled in moist sand at 5 C for 5 weeks to stimulate germination. Following this, seeds were placed in light/dark or dark conditions at 13/7 C. Cold stratification, caused seeds to germinate even in the absence of incubation at 13/7 C. Complete (100%) germination occurred within 4 weeks of cold stratification at 5 C (Table 3.6). The seeds that did not germinate after 1 to 3 weeks of cold stratification were transferred to 13/7 C temperature cabinet in the light/dark and dark regimes.

In the absence of chilling, seed germination only occurred in the dark. However, the chilling treatment enabled some seed germination to take place even in the light/dark treatment. It is known that seeds of some species require a period of chilling, whilst in the imbibed state, to stimulate germination. For example, germination promoted by cold stratification in catchweed bedstraw has been reported previously (Grime et al. 1981; Slade and Causton 1979). Temperature lower than 5 C often occurs in the southern Australian grain belt in winter (June to August) and this periodic cold stratification may be important for germination of threehorn bedstraw. It is also

noteworthy that cold stratification was effective in the dark. While in the fields, seeds may be present on the soil surface, so their response could be different to that observed here.

**Table 3.6.** Effects of cold stratification on seed germination of the RC population. During cold stratification (5 C), seeds were placed in the Petri dishes containing moist sand and every week seeds were exhumed from the sand and ungerminated seeds were placed on filter papers at 13/7 C in both light/dark and dark conditions. Germination at 13/7 C was examined after 14 days of incubation and represented as percent of the ungerminated seeds.

Time	Germination				
	5 C	Sequential treatment for 14 days at 13/7 C			
		Light/dark Dark			
—week—		%			
0	0.0	0.0	35.0		
1	15.0	23.3	35.0		
2	60.0	8.3	15.0		
3	88.3	2.8	2.8		
4	100.0	-	-		
5	100.0	-	-		
LSD 0.05	—4.3—	19.6			

In order to further study the effects of cold stratification, seeds were incubated with or without KNO<sub>3</sub> (0.005 M) at 13/7 C and 5 C for 1 to 4 weeks. Only 17% of seeds moistened with water germinated at 13/7 C, even after 4 weeks of incubation (Figure 3.14). In contrast, treating seeds with KNO<sub>3</sub> increased germination to 47% after 1 week of incubation. Cold stratification increased germination and germination was greater with KNO<sub>3</sub> than without KNO<sub>3</sub> after 2 and 3 weeks of incubation. However, after 4 weeks of incubation, there were no differences between these treatments. Statistically the differences in germination rates between treatments, times and temperature were significant (P < 0.01) as was the interaction between most of these factors (Table 3.7). Only the interaction of all three factors (temperature by time by KNO<sub>3</sub>) was non-significant. This experiment clearly demonstrates that germination in this species is responsive to both cold stratification and KNO<sub>3</sub>.



Figure 3.14. Interaction effect of temperature and KNO<sub>3</sub> on germination of threehorn bedstraw (RC population) seeds when incubated in darkness for different periods of time. Seeds were placed at 5 C (triangle) and 13/7 C (circle) with (open symbols) and without (solid symbols) 0.005 KNO<sub>3</sub>. Vertical Μ bars represent standard errors.

**Table 3.7.** Degrees of freedom (df) and level of significance for temperature (13/7 C and 5 C), time (1 to 4 weeks),  $KNO_3$  (0.005 M or absence) and their interactions on seed germination of the RC population of threehorn bedstraw.

Factor	df	F Probability
Temperature	1	<i>P</i> = 0.01
Time	3	<i>P</i> < 0.001
KNO <sub>3</sub>	1	<i>P</i> < 0.001
Temperature by time	3	<i>P</i> < 0.001
Temperature by KNO <sub>3</sub>	1	<i>P</i> < 0.001
Time by KNO <sub>3</sub>	3	<i>P</i> = 0.01
Temperature by time by KNO <sub>3</sub>	3	NS

Residual mean square = 65.03 with 30 df.

## 3.3.5.2 Small-Flowered Mallow

Seed germination of small-flowered mallow was not stimulated by the chilling treatment at 5 C (data not shown). This suggests that vernalisation is not required to break the seed coat-imposed dormancy in small-flowered mallow. However, the coat-imposed dormancy in other weed species can be broken by the chilling treatment (e.g. Webb and Wareing 1972).

# 3.3.6 Effect of Salt and Osmotic Stress on Germination

### 3.3.6.1 Salt Stress

Seed germination of threehorn bedstraw was inversely related to NaCl concentration (Figure 3.15). The data were best fitted to a quadratic polynomial equation ( $y = 0.0017x^2 - 0.574x + 50.326$ ,  $r^2 = 0.999$ ). Germination was greater than 44% at less than 20 mM NaCl and was lowest (2%) at 160 mM NaCl. Some seeds of texasweed [*Caperonia palustris* (L.) St. Hil.] were also germinated at the concentration of 160 mM NaCl (Koger et al. 2004).

A three-parameter logistic model {G (%) =  $98.1/[1 + (x/89.6)^{4.3}]$ ,  $r^2 = 0.999$ } was fitted to the germination data of common sowthistle seeds at different concentrations of NaCl (Figure 3.15). Germination was greater than 90% at 40 mM NaCl and some germination occurred even at 160 mM NaCl (7.5%). However, germination was completely inhibited at 320 mM NaCl. The concentration for 50% inhibition of the maximum germination, estimated from the fitted model, was 89.6 mM NaCl.

Germination of wild turnip seeds was influenced (P < 0.01) by the interaction between light and NaCl concentration (Figure 3.15). Germination in dark was greater than in light/dark at all the concentrations of NaCl, except at 0 and 320 mM. In the dark, seed germination was relatively unaffected (53 to 60%) at a low level of salinity (80 mM NaCl) and some germination occurred even at 160 mM NaCl (18%). However, germination was completely inhibited at 320 mM NaCl. These data suggest that even at high soil salinity, a small proportion of wild turnip seeds may germinate. On the other hand, germination of wild turnip seeds was significantly reduced by the exposure to light even at 10 mM NaCl and completely inhibited at concentrations greater than 80 mM. This interaction suggests that the combination of NT and salinity could have a major impact on germination of wild turnip seeds.



**Figure 3.15.** Effect of sodium chloride (NaCl) concentration on germination of YP population of threehorn bedstraw (at 13/7 C in dark), common sowthistle and E5 population of annual ryegrass (at 20/12 C in light/dark), wild turnip (at 20/12 C in light/dark and dark), Indian hedge mustard and small-flowered mallow (at 25/15 C in light/dark). The lines represent the quadratic polynomial (threehorn bedstraw), three-parameter logistic (common sowthistle and Indian hedge mustard), exponential decay curve (small-flowered mallow) and linear model (annual ryegrass) fitted to the data.

A three-parameter logistic model {G (%) =  $51.96/[1 + (x/67.51)^{3.68}]$ , r<sup>2</sup> = 0.996} was fitted to the germination percentage of Indian hedge mustard seed at different concentrations of NaCl (Figure 3.15). There was no change in seed germination up to 20 mM NaCl concentration (51 to 53%), but then germination decreased sharply with increased NaCl concentration, and no germination occurred at 160 mM NaCl. The concentration of NaCl required for 50% inhibition of the maximum germination (*x*<sub>50</sub>), estimated from the fitted model, was 67.5 mM (Figure 3.15).

Germination of small-flowered mallow seeds was inversely related to NaCl concentration ( $y = 54.64 \times e^{-0.02x}$ ,  $r^2 = 0.986$ ); however, some seeds germinated at all concentrations to 160 mM NaCl (Figure 3.15). Germination was greatest (58%) at 0 mM NaCl and was lowest (2%) at 160 mM NaCl. Round-leaved mallow, a related species, has been reported as salt tolerant in Europe (Makowski and Morrison 1989).

Germination of annual ryegrass seeds decreased linearly [G(%) = 58.89 - 0.19x, r<sup>2</sup> = 0.984] as NaCl concentration increased from 0 to 320 mM (Figure 3.15). Seed germination was greater than 50% up to NaCl concentration of 40 mM and some germination occurred even at 160 mM NaCl. However, germination was completely inhibited by 320 mM NaCl. The results for the E5 population of annual ryegrass differ from the findings of Marcar (1987), who reported no reduction in germination from 0 to 200 mM NaCl for the 'Wimmera' cultivar of annual ryegrass. It is possible that the 'Wimmera' cultivar may be more tolerant to salt than the wild population used in this study.

The NaCl concentration required for 50% inhibition of the maximum germination was relatively higher for common sowthistle, wild turnip (in the dark) and annual ryegrass compared to the other weed species in this study (Table 3.8). Saline soil types are common in southern Australia, and few seeds of these species may germinate at high soil salinity. On the other hand, germination of threehorn bedstraw, wild turnip (on the soil surface), Indian hedge mustard and small-flowered mallow may be inhibited more by soil salinity compared to the other weed species (Table 3.8).

Weed species	NaCl		
	mM		
Threehorn bedstraw	54		
Common sowthistle	90		
Wild turnip-light/dark	8		
Wild turnip-dark	135		
Indian hedge mustard	68		
Small-flowered mallow	32		
Annual ryegrass	148		

**Table 3.8.** Concentrations of sodium chloride (NaCl) required for 50% inhibition of the maximum germination of different weed species. The concentrations were estimated from the fitted model or visually (where model was not fitted).

# 3.3.6.2 Osmotic Stress

Germination of threehorn bedstraw decreased linearly (G = -72.98x + 54.155, r<sup>2</sup> = 0.976) as osmotic potential decreased from 0 to -0.8 MPa (Figure 3.16). Germination was completely inhibited at an osmotic potential of -0.8 MPa; however, 4% of seeds were still able to germinate at -0.6 MPa.

A three-parameter logistic model {G (%) =  $98.11/[1 + (x/0.32)^{-0.12}]$ ,  $r^2 = 0.990$ } was fitted to the germination percentage of common sowthistle seed at different osmotic potential (Figure 3.16). Germination decreased from 95% to 11% as osmotic potential decreased from 0 to -0.6 MPa. Germination was completely inhibited at osmotic potential of -0.8 MPa or lower. While common sowthistle is quite sensitive to osmotic potential, some seeds can germinate under marginal water stress conditions. The osmotic potential for 50% inhibition of the maximum germination, estimated from the fitted model, was -0.32 MPa.

Similar to the response to NaCl, seed germination of wild turnip was affected by the interaction (P < 0.001) between light and osmotic potential (Figure 3.16). Seed germination was greater in dark than in light/dark at all osmotic potential treatments, except at 0 MPa. Seed germination was significantly reduced by exposure to light, even at an osmotic potential of -0.1 MPa, indicating extreme sensitivity to the stress

when exposed to light. Under light/dark conditions, germination was completely inhibited at osmotic potential of -0.8 MPa. Under dark conditions, there was little decline in germination up to the concentration of -0.6 MPa (44 to 54%), and then germination decreased as osmotic potential decreased from -0.6 MPa. However, 8% seeds were still able to germinate at an osmotic potential of -1.0 MPa, which indicates that some seeds of this species can germinate under moderate water stress. Such conditions can occur temporarily for buried seeds in between rainfall events at the start of the growing season in southern Australia.

An exponential decay curve [G (%) = 52.94 x e<sup>-5.1x</sup>, r<sup>2</sup> = 0.982] was fitted to the germination percentage of Indian hedge mustard seeds at different osmotic potential (Figure 3.16). The maximum germination ( $G_{max}$ ) estimated from the fitted model was 53% and germination decreased sharply as osmotic potential decreased from 0 to – 1.0 MPa. Germination was completely inhibited at osmotic potential of –0.8 MPa or lower.

Germination of small-flowered mallow seeds decreased as osmotic potential decreased from 0 to -1.0 MPa (Figure 3.16). Germination was completely inhibited at osmotic stress lower than -0.4 MPa, which indicates that seeds are moderately sensitive to low water potential. The seedling emergence of a related weed, round-leaved mallow, also decreased below the soil water potential of -0.28 MPa (Blackshaw 1990).

Germination of annual ryegrass seeds decreased linearly [G(%) = 62.24 - 51.30x, r<sup>2</sup> = 0.989] as the osmotic potential of the germination medium decreased from 0 to – 1.0 MPa (Figure 3.16). However, even at an osmotic potential of –1.0 MPa, about 8% of seeds germinated. In general, germination of annual ryegrass seeds is inhibited under water stress conditions. Failure to germinate under such conditions could be a valuable strategy for annual ryegrass survival to alternating wetting and drying of the soil.



**Figure 3.16.** Effect of osmotic potential on seed germination of YP population of threehorn bedstraw (at 13/7 C in dark), common sowthistle and E5 population of annual ryegrass (at 20/12 C in light/dark), wild turnip (at 20/12 C in light/dark and dark), Indian hedge mustard and small-flowered mallow (at 25/15 C in light/dark). The lines represent the linear (threehorn bedstraw and annual ryegrass), three-parameter logistic (common sowthistle) and exponential decay curve model (Indian hedge mustard) fitted to the data.

Germination in trumpetcreeper [*Campsis radicans* (L.) Seem. *ex.* Bureau] was completely inhibited at osmotic potentials lower than -0.2 MPa (Chachalis and Reddy 2000). Similarly, seeds of redvine [*Brunnichia ovata* (Walt.) Shinners] were highly sensitive to low water potential (Shaw et al. 1991). These comparisons suggest that threehorn bedstraw, common sowthistle, wild turnip (in the dark) and

annual ryegrass have some capacity to germinate under moisture stress conditions, which can occur temporarily in southern Australia between the rainfall events at the start of the growing season. Other more water stress tolerant weed species such as texasweed have been shown to be able to germinate at low percentages (9%) even at a water potential of -0.8 MPa (Koger et al. 2004). Similarly, seeds of London rocket germinated up to an osmotic potential of -1.2 MPa (Ray et al. 2005). In contrast, seeds of small-flowered mallow and Indian hedge mustard are not very tolerant to water stress and are likely to be favoured by a moist environment. Makowski and Morrison (1989) reported that major infestations of round-leaved mallow, a weed related to small-flowered mallow, generally occur in regions of western Canada where relative precipitation levels are high.

Salinity is a major abiotic constraint for plants and can negatively affect important physiological processes (DiTommaso 2004; Greenway and Munns 1980). In addition to physiological processes in the plants, sodium present in salt can modify soil structure and fertility by replacing calcium and magnesium in the anion exchange process and this leads to nutrient and water stress (DiTommaso 2004). Although in this study the effect of salt and osmotic stress on seed germination was studied separately, the combination of these two factors in the field could have adverse effects on seed germination of these weed species. Further research is needed under field situations where different soil texture and temperature may affect the soil moisture and thereby germination and seedling establishment.

# 3.3.7 Effect of Age and Depth on the Fate of Seed

### 3.3.7.1 Threehorn Bedstraw

Before burial in the field, germination of both populations (RC and YP) of threehorn bedstraw seeds was extremely low (< 5%). After this period (in mid April), twothirds of the seeds were buried at 2 and 5 cm and the other one-third remained on the soil surface. Results of ANOVA (after seed burial) are summarised in Table 3.9.

Regardless of the population, seed germination increased with the depth of seed burial (Figure 3.17). After 1.5 months of burial, germination of seeds that were

buried at 5 cm was 44% and 57% for the RC and YP populations, respectively. Germination of the surface placed seeds was negligible (< 2%). After 3 months of seed burial (in July), germination of both populations increased to the maximum level, regardless of burial depth. Germination of the RC population was 43, 65 and 88% for seeds after-ripened at 0, 2 and 5 cm, respectively. While for the YP population, germination of seeds after-ripened at 0, 2 and 5 cm was 47, 72 and 93%, respectively. During subsequent months (until 7.5 months after seed burial), germination of both populations of threehorn bedstraw seeds declined regardless of the burial depth. Generally, seed germination of threehorn bedstraw showed only one peak germination period. Similar germination periodicity has been reported for several other weed species that display cyclic dormancy (Baskin and Baskin 1985, 1987; Mennan 2003; Mennan and Ngouajio 2006; Omami et al. 1999).

Source of variation	df	F-values
Population	1	< 0.001
Seed age	4	< 0.001
Depth	2	< 0.001
Population by seed age	4	< 0.001
Population by depth	2	0.006
Seed age by depth	8	< 0.001
Population by seed age by depth	8	0.122
Error	58	

**Table 3.9.** ANOVA results for population, seed age and depth of seed burial and their interactions on germination of threehorn bedstraw seeds<sup>a</sup>.

<sup>a</sup>Abbreviations: df, degree of freedom.

Laboratory germination of the surface placed seed was lower than the buried seed. It appears that the buried seed lost dormancy faster than the surface seed. Evidence from an earlier experiment has shown that light inhibits germination in this species (section 3.3.1.1). Secondary dormancy was induced in the seeds of catchweed bedstraw, a closely related species, when seeds were continuously exposed to light (Malik and Vanden Born 1987b). Therefore, we could speculate that light might have induced dormancy in the surface after-ripened seeds of this species. Seeds of

threehorn bedstraw showed greatest germinability during the coldest part of winter (Table 3.2), which may be due to the release of dormancy via cold stratification. The cold stratification experiment has shown that cold temperature stimulated seed germination of this species (section 3.3.5.1).



**Figure 3.17.** Effect of seed age and burial depth on germination of RC (a) and YP (b) populations of threehorn bedstraw seeds. Germination was tested by incubating seeds at 13/7 C (12 h/12 h) in darkness for 14 days.

Lower seed germination for the surface seed compared to the buried seed suggests that lower germination of this species may be expected under NT systems where most of the weed seeds remain on the soil surface after the sowing operation. On the other hand, greater germination may be expected under traditional cultivation systems where most of the weed seeds are likely to be buried by tillage. Stimulation of seedling emergence by tillage has been reported in false cleavers and catchweed bedstraw (Reid and Van Acker 2005). The information on germination pattern could be used in integrated weed management programs to time sowing so that crop establishment is achieved before peak emergence of threehorn bedstraw seedlings. However, late seedling emergence of this species could allow some seedlings to

escape the application of pre-sowing herbicides and lead to a replenishment of the seed bank.

## 3.3.7.2 Common Sowthistle

Seeds of common sowthistle were initially after-ripened on the soil surface for 3 months. During this time, seed germination was not observed in the field probably because of low soil moisture. After burial of seeds, comparisons between seed burial depth over time (months after seed burial) were made for different components of seed fate (germination in the field and laboratory, decayed and dormant seed). Germination in the field was only influenced by the depth of seed burial (Table 3.10) and was not observed in seeds buried at 5 cm (Figure 3.18). Lack of germination at depth could be due to dormancy enforced by darkness. It could also be due to the fatal germination of buried seed as only the established plants were counted, as it was impossible to differentiate between fatal germination and seed decay.

Seed decay was significantly influenced (P < 0.001) by the interaction between seed age and depth of burial (Table 3.10 and Figure 3.18). Early in the season (1.5 months after seed burial), seed decay was greater in the buried seed compared to the surface seed. However, at the end of the study (7.5 months after seed burial), seed decay was greater for the seeds after-ripened on the soil surface (82%) compared to the seeds buried at 2 cm (62%) or 5 cm (55%). Seed decay increased continuously with time for the seeds after-ripened on the soil surface. For the buried seed, this component was found to increase only up to 3 months of seed burial.

The continuous increase in decay of the seeds on the soil surface was probably due to their exposure to the most variable fluctuations (extremes) in environmental conditions which could promote metabolic failure (e.g. Taylorson 1970). In contrast, buried seed are likely to persist longer due to dark enforced dormancy (created by burial) in a large portion of the seed bank. In addition, the possibility of microbial decay cannot be ruled out. On or near the soil surface, seeds not only face greater risk of loss through predation or by microorganisms and pathogens, but they also decay more rapidly and mortality rate is increased by germination in unfavourable environments (Ballare et al. 1988; van Esso et al. 1986). The results of the experiment suggest that farming practices that allow a large proportion of seeds to remain on or near the soil surface will promote quicker depletion of the seed bank in the absence of seed replenishment. The dormant component of the seed bank was small and appeared to be unaffected by the depth of burial.

Source of variation	df	SS	F-values
		Field germin	ation
Seed age	4	61	0.488
Depth	2	458	< 0.001
Seed age by depth	8	42	0.958
Error	28	486	
		Decayed se	eed
Seed age	4	8598	< 0.001
Depth	2	1030	0.01
Seed age by depth	8	7092	< 0.001
Error	28	2640	
		Lab germind	ation
Seed age	4	7686	< 0.001
Depth	2	3388	< 0.001
Seed age by depth	8	5718	0.001
Error	28	4386	
		Dormant s	eed
Seed age	4	264	0.234
Depth	2	81	0.414
Seed age by depth	8	202	0.796
Error	28	1249	

**Table 3.10.** ANOVA results for effect of seed age and/or burial depth on seed fate of common sowthistle<sup>a</sup>.

<sup>a</sup>Abbreviations: df, degree of freedom; SS, sum of squares.



Figure 3.18. Effect of age and burial depth on the fate of common sowthistle seeds. Seeds were after-ripened at 0, 2 5 cm. Different and components of seed fate were laboratory germination (white), field germination (horizontal line), decayed seed (vertical line) and dormant seed (diagonal line).

Fresh seeds of common sowthistle used in this study had very little innate (primary) dormancy, resulting in 93% germination in the laboratory (data not shown). Laboratory germination of common sowthistle seeds before burial in the field (until 3 months from seed after-ripened on the soil surface) was always greater than 90% (data not shown). Germination in the laboratory (after burial of seeds) was significantly influenced by the interaction (P < 0.01) between seed age and depth of seed burial (Table 3.10; Figure 3.18). After 1.5 months of seed burial, germination in the laboratory was 82, 65 and 59% for the seeds after-ripened at 0, 2 and 5 cm,

respectively. At the last exhumation time (7.5 months after seed burial), germination in the laboratory was lower for the seeds after-ripened on the soil surface (8%) compared to the buried seed (32 to 42%). This lower laboratory germination of the seeds on soil surface was mainly due to greater seed decay and fatal germination for surface seed compared to the buried seed.

At the end of the growing season, 32 to 42% of the seeds were still viable at depth (2 and 5 cm) and germinated readily in the laboratory under light/dark conditions. It is possible that buried seed of common sowthistle persist due to the absence of light, which is supported by the result of the temperature and light experiment where germination was much lower in the dark. In contrast, persistence of a smaller fraction (8%) on the soil surface for extended periods could be due to rapid drying and unfavourable conditions for germination.

## 3.3.7.3 Wild Turnip

Seeds of wild turnip were also initially after-ripened on the soil surface for 3 months. During this time, seed germination was not observed in the field probably because of low soil moisture due to lack of rainfall. After 3 months, two-thirds of the bags were buried at 2 and 5 cm and the other one-third remained on the soil surface. Germination in the field was influenced only by the depth of seed burial (Table 3.11) and was observed only in the surface seed (5 to 12%; Figure 3.19). Lack of germination in the buried seed could be due to the fatal germination of buried seed, as only the established plants were counted because it was impossible to differentiate between fatal germination and seed decay. It could also be due to presence of the nylon bags influencing seed behaviour.

Regardless of seed burial depth, the decay component in wild turnip seeds was very high and found to increase only up to 3 months of seed burial. At the end of the growing season (7.5 months of seed burial), the decay component was similar between seeds after-ripened at various depths and ranged from 77 to 87%. It appears that the seed decay may be the main contributor to the loss of seeds of this species from the seed bank. Seed decay in the surface seed was most likely due to their exposure to the most variable fluctuations in environmental conditions, which could

promote metabolic failure (e.g. Taylorson 1970). While high seed decay in the buried seed could be due to fatal germination (seeds might have germinated in the field but would have failed to establish into seedlings). In spite of different burial depths, the level of seed dormancy was similar (P > 0.05) between seeds after-ripened at different depths (Table 3.11 and Figure 3.19). However, 12 to 18% of the seeds were still dormant at the end of the growing season (7.5 months after seed burial).

Source of variation	df	SS	F-values
		Field germine	ation
Seed age	4	24	0.235
Depth	2	751	< 0.001
Seed age by depth	8	49	0.209
Error	28		
		Decayed se	ed
Seed age	4	6070	< 0.001
Depth	2	1068	0.005
Seed age by depth	8	877	0.275
Error	28		
		Lab germina	tion
Seed age	4	6791	< 0.001
Depth	2	34	0.645
Seed age by depth	8	632	0.077
Error	28		
		Dormant se	eed
Seed age	4	81	0.708
Depth	2	124	0.209
Seed age by depth	8	159	0.825
Error	28	1052	

**Table 3.11.** ANOVA results for effect of seed age and/or depth on fate of wild turnip seeds.

<sup>a</sup>Abbreviations: df, degree of freedom; SS, sum of squares.

Germination of fresh seeds of wild turnip was 58% in the laboratory, which indicates that the seeds had a marginal level (36%) of innate dormancy (Table 3.12). However, germination increased to 70% after 3 months of seed storage. This increase in germination was due to decrease in seed dormancy (Table 3.12). After burial of seeds, germination in the laboratory was influenced only by seed burial duration (Table 3.11; Figure 3.19), which was mainly due to the high level of seed decay and fatal germination rather than induction or enforcement of dormancy.



**Figure 3.19.** Effect of age and burial depth on the fate of wild turnip seeds. Seeds were after-ripened at 0, 2 and 5 cm. Different components of seed fate were laboratory germination (horizontal line), field germination (vertical line), decayed seed (white) and dormant seed (diagonal line).

Components of seed		LSD 0.05		
fate	0	1.5	3	
	-months after			
Decayed seed (%)	6.7	5.0	5.0	NS
Dormant seed (%)	35.6	30.0	25.0	NS
Lab germination (%)	57.8	65.0	70.0	NS

**Table 3.12.** Fate of wild turnip seeds before burial at different depths in the field<sup>a</sup>.

<sup>a</sup>Abbreviations: NS, non-significant.

# 3.3.7.4 Annual Ryegrass

In 2005, the fate of annual ryegrass seeds was investigated for five different populations collected from different fields on the same farm. The different components of seed fate (laboratory and field germination, decayed and dormant seed) measured were not influenced (P > 0.05) by the interaction between population and seed age or by the population. Therefore, the data was pooled across the populations.

Less than 2% of fresh seeds germinated, regardless of population. This indicates that a high proportion of seeds were dormant at the beginning of the experiment (Figure 3.20). After 4.5 months of after-ripening in the field, seed germination reached 60%. However, after this time the contribution of laboratory germination to the seed fate started to decline. This decline in laboratory germination was most likely due to seed decay and germination in the field. The seed decay component increased with time and the dormant seed component decreased with time (Figure 3.20). After 10.5 months of after-ripening in the field, the seed decay component was 54% of the total. The level of seed dormancy declined from 95% at the start of the experiment to around 19% after 10.5 months of after-ripening.

Although annual ryegrass is known to possess high genetic variability, populations collected from the same farm showed no variation in the level of seed dormancy (P = 0.37). Differences in dormancy between seed lots can be influenced by the environmental conditions the mother plants are exposed to such as precipitation, altitude, temperature and soil moisture (Andersson and Milberg 1998; Baskin and

Baskin 1998; Meyer and Monsen 1992; Philippi 1993). However, these conditions are likely to be similar for populations collected on a single farm.

Seeds after-ripened dry in a glasshouse behaved very differently to seeds afterripened in the field. Dormancy was released in seeds after-ripened in the field earlier than for seeds after-ripened in the glasshouse. After 3 months of storage, 44% seeds were dormant when after-ripened in the field, but 84% seeds were still dormant when after-ripened dry in the glasshouse. This impact of after-ripening environment on dormancy release was probably caused by lower levels of moisture in the seeds afterripened in the glasshouse as drier seeds have been found to lose dormancy more slowly than wetter seeds (Steadman et al. 2003b). The results show that conclusions drawn from an artificial storage condition (e.g. dry in a glasshouse) may not be useful for interpreting behaviour in the field.



Figure 3.20. Fate of annual ryegrass seeds in 2005 when after-ripened on the soil surface in a field (a) and in a glasshouse (b). Data for five populations were pooled, as the interaction between population and seed age, and effects of population non-significant. were Different components of seed fate were laboratory germination (horizontal line), field germination (vertical line), decayed seed (white) and dormant seed (diagonal line).

In 2004, seeds were after-ripened on the soil surface with or without crop residue, or buried at 5 cm without residue. The different components of seed fate of these samples were measured and the results of the ANOVA are summarised in Table 3.13. Germination in the laboratory was influenced only by the after-ripening duration (P < 0.001) indicating a change in germinability with seed age (Figure 3.21). Germination of fresh seed was 40%, which indicates a high proportion of dormancy at the start of the experiment. Regardless of the seed after-ripening environment, germination exceeded 70% after 3 months. However, after May, the contribution of laboratory germination to seed fate started to decline. This was most likely due to seed decay and germination in the field.

Seed germination in the field was influenced by the interaction between seed age and after-ripening environment (Table 3.13), reflecting greater field germination of buried seed as well as of surface seed in the presence of crop residue (Figure 3.21). Seed germination in the field was only observed between 4 to 7 months. After this period, it was difficult to distinguish between decayed seeds or seeds that decayed after germinating in the field (e.g. Davis et al. 2005); therefore it was assumed that no further field germination occurred after 7 months (August).

Germination in the field was generally greater for the seeds buried at 5 cm than for seeds after-ripened on the soil surface in the presence of crop residue. Germination of the latter was greater than for seeds after-ripened on the soil surface but without crop residue. The only exception to this general trend was observed right at the onset of field germination (at 4 months of after-ripening) when germination was greater for the seeds after-ripened on the soil surface with crop residue (15%) compared to the seeds buried at 5 cm (2%). The greater initial field germination of seeds after-ripened on the soil surface with crop residue to greater available soil moisture than in the absence of crop residue and for seeds at 5 cm depth. At this early period of the growing season, moisture may not have reached 5 cm deep. The final germination in the field was 38, 24 and 12% for the seeds after-ripened at 5 cm depth, on the soil surface with crop residue and on the soil surface without crop residue, respectively. Mohler and Galford (1997) also found lower field germination. In

contrast, seeds buried at a shallow depth in the soil may have been exposed to more favourable moisture and temperature conditions, which would stimulate germination.

Source of variation	2004-05		2005			
	df	SS	F-Values	df	SS	F-Values
			Field gern	ninatior	ı	
Age	8	10879	< 0.001	7	4696	< 0.001
AR Environment	2	1640	< 0.001	2	6192	< 0.001
Age by AR environment	16	2360	< 0.001	14	983	< 0.001
Error	52	594		46	112	
			Decayed	d seed		
Age	8	16832	< 0.001	7	9591	< 0.001
AR Environment	2	1628	< 0.001	2	5637	< 0.001
Age by AR environment	16	1737	< 0.001	14	1431	< 0.001
Error	52	626		46	415	
			Lab germ	ination		
Age	8	20874	< 0.001	7	7582	< 0.001
AR Environment	2	48	0.565	2	1856	< 0.001
Age by AR environment	16	1045	0.112	14	2119	< 0.001
Error	52	2164		46	1085	
			Dorman	t seed		
Age	8	39871	< 0.001	7	6485	< 0.001
AR Environment	2	61	0.474	2	2149	< 0.001
Age by AR environment	16	1176	0.054	14	1616	0.001
Error	52	2102		46	1612	

**Table 3.13.** ANOVA results for effects of seed age and after-ripening (AR) environment on seed fate of annual ryegrass in 2004-05 and 2005<sup>a</sup>.

<sup>a</sup>Abbreviations: df, degree of freedom; SS, sum of squares.



Figure 3.21. Effect of age and after-ripening environment on the fate of annual ryegrass seeds in 2004-05. Seeds were afterripened on the soil surface without residue (a), with residue (b), and buried at 5 cm without residue (c). Different components of seed fate were laboratory germination (horizontal line). field germination (vertical line), decayed seed (white) and dormant seed (diagonal line).

Seed decay, another important component of seed fate, was also influenced by the interaction between seed age and after-ripening environment (Table 3.13). Regardless of seed after-ripening environment, seed decay was found to increase with seed age (Figure 3.21). Before 4 months of seed after-ripening, the seed decay component was small and similar between the seeds in the different environments. After this time, decay was always greater for the seeds after-ripened on the soil surface without residue followed by seeds after-ripened on the soil surface with

residue and then seeds buried 5 cm deep. After 16 months, the seed decay component was 57, 44 and 32% for these three after-ripening environments. As mentioned earlier, greater seed decay on the soil surface was probably due to exposure to greater fluctuations in environmental conditions.

The component of the seeds remaining dormant was also influenced (P = 0.05) by the interaction between seed age and after-ripening environment (Table 3.13). This interaction was mainly due to the later release of dormancy for buried seed compared with seeds on the soil surface, regardless of the presence of residue. However, later in the season the level of dormancy was similar between seeds in the different environments. Irrespective of the seed after-ripening environment, all seeds lost dormancy completely after 16 months of storage.

In 2005, seeds of the E5 population were initially after-ripened on the soil surface for 4 months and then two-thirds of the seeds were buried (in May) at 2 and 5 cm to simulate tillage. Germination in the laboratory was not observed for fresh seeds in this year, which was different to the previous year (2004) when germination of fresh seeds was 40%. This indicates that the level of dormancy in fresh seeds was greater in 2005 than 2004. Differences in weed seed germination at maturity among seeds collected in different years has been shown previously for other weed species (Andersson and Milberg 1998; Beckstead et al. 1996). Factors such as rainfall (Philippi 1993), soil moisture and temperature (Peters 1982) during seed maturation or the nutritional status of the mother plant (Fawcett and Slife 1978; Watson and Watson 1982) could affect seed germination (dormancy) in different years.

In this study, seeds sampled at 2, 3 and 4 months of after-ripening on the soil surface had 9, 13 and 47% germination when incubated in the laboratory. Germination was not observed in the field during this period due to low soil moisture conditions. The results of the ANOVA for 2005 are summarised in Table 3.13. In 2005, all the components of seed fate (germination in the laboratory and field, decayed and dormant seed) were influenced by the interaction between seed age and after-ripening environment. Regardless of seed after-ripening environment, the laboratory germination component was greater than 45% at 1 month after seed burial (June), but after that it started to decrease as the growing season progressed (Figure 3.22). This

was most likely due to seed decay or germination of seeds in the field. Seed germination in the field was observed at 1 month after seed burial and was always lower for the surface seed compared with seed buried at 2 or 5 cm.

Similar to the experiment 2004-05, the seed decay component in 2005 was found to increase and the dormant seed component decreased with time (Figure 3.22). The seed decay was always greater for seeds after-ripened on the soil surface, which is consistent with the experiment 2004-05. After 8 months of seed burial, the seed decay component was 56, 29 and 37% for the seeds after-ripened at 0, 2 and 5 cm, respectively. Lower seed decay at 2 cm compared with 5 cm was offset by greater field germination at 2 cm. Again, greater seed decay on the soil surface (0 cm) was probably due to exposure to greater fluctuation in environmental conditions that could promote metabolic failure (e.g. Taylorson 1970).

In these studies, mesh bags were used to after-ripen seeds in the field. In a recent study, Van Mourik et al. (2005) found that buried mesh bags might overestimate decay rates of soil seed banks. However, decreasing the density of weed seeds in bags significantly reduced seed decay, most probably because of decreased seed-to-seed contamination by pathogenic fungi. This problem was not evident at seed densities less than 10000 seeds/m<sup>2</sup>. In the present study, the seed density of all weed species in mesh bags was much lower than the critical density of Van Mourik et al. (2005) and is not likely to have affected the results.



Figure 3.22. Effect of seed and after-ripening age environment on the fate of annual ryegrass seeds of E5 population in 2005. Seeds were after-ripened on the soil surface (a), buried at 2 cm (b), and buried at 5 cm (c). Different components of seed fate were laboratory germination (horizontal line), field germination (vertical line), decayed seed (white) and dormant seed (diagonal line).

In 2005, seeds buried at 2 and 5 cm released dormancy earlier than the seeds afterripened on the soil surface. Similar to the experiment 2004-05, seed dormancy was not a major component of the seed fate. At the last sampling period, the level of dormancy was quite small but similar (P > 0.05) between the after-ripening environments, ranging from 0 to 7% (Figure 3.21 and 3.22).

Although seed after-ripening environments and sampling frequency were slightly different between the years (2004-05 and 2005), the results show consistency in the fate of annual ryegrass seeds. In both years, seed decay was found to be an important component of seed fate, particularly for the seeds after-ripened on the soil surface. This suggests that the farming practices that allow a large proportion of seeds to remain on the soil surface will promote quicker depletion of the seed bank provided there is no seed replenishment. In contrast, farming practices that achieve shallow burial of seeds will promote greater emergence of seedlings.

Although the dormant component in the fresh seeds of annual ryegrass was different between years, dormancy declined rapidly in all treatments to be quite small at the end of the growing season. At the end of the growing season 28 to 36% of the seeds across all treatments were still viable, and germinated readily in the laboratory (Figures 3.21 and 3.22). Previous research on annual ryegrass identified a 'dark dormant' component of the seed bank that only germinates when exposed to light (Gramshaw and Stern 1977). It is possible that buried seed of annual ryegrass persist due to absence of light. In contrast, seeds on the soil surface persist for extended periods due to rapid drying and unfavourable conditions for germination.

Inferences drawn from the results of this study should be limited to the weed populations sampled because weed ecotypes often vary in dormancy characteristics and germination requirements. Seed germination behaviour may also differ among seeds produced in different years. Therefore, inferences are most relevant to the southern Australian weed populations tested. Further research is needed on the influence of weather and soil conditions on the seed fate of these weed species.

# 3.3.8 Effect of Seed Burial Depth on Seedling Emergence

## 3.3.8.1 Threehorn Bedstraw

Seedling emergence of threehorn bedstraw was influenced by burial depth (Figure 3.23). Seedling emergence increased with shallow burial but decreased with deeper seed burial. Seedling emergence was greatest for seeds buried at 1 to 2 cm (89 to 91%). Seedlings were able to emerge from the depth of 5 cm (81%); however, it was

lower than the emergence that occurred at 1 and 2 cm. No seedlings emerged from seeds placed on the soil surface (0 cm) or from seed buried at 12 cm.

Light might have inhibited germination of threehorn bedstraw on the soil surface. Germination of two closely related species, catchweed bedstraw and false cleavers, has also been reported to be inhibited by light (Malik and Vanden Born 1988). Similar findings have been presented earlier in this chapter (see section 3.3.1) for threehorn bedstraw. Boyd and Van Acker (2003) also found greater seedling emergence of catchweed bedstraw at the shallow burial depth of 1 to 2 cm. The seedling emergence behaviour of seeds buried at 12 cm depth may be linked to inadequate seed energy reserves (Mennan and Ngouajio 2006). The results suggest that the farming practices that achieve shallow burial of seeds will promote seedling emergence of threehorn bedstraw.

## 3.3.8.2 Small-Flowered Mallow

Seedling emergence of scarified seeds of small-flowered mallow was markedly influenced by the burial depth (Figure 3.23). Only 35% of seeds produced seedlings when sown on the soil surface. Seedling emergence increased with shallow burial at depths of 0.5 to 2 cm (60 to 62%), but progressively declined as depth increased. Light is not inhibitory for seed germination in this species (section 3.3.1.5), so lower seedling emergence on the soil surface was probably due to the lower availability of moisture. No emergence was recorded from 8 or 10 cm. Although the cause of low or no emergence from deep buried seed was not investigated, it could be due to fatal or no germination. Blackshaw (1990) reported similar results for round-leaved mallow, with the greatest emergence occurring at depths of 0.5 to 2 cm, with emergence declining significantly from 3 to 6 cm and no emergence occurring at 8 cm. Inability to emerge from deep burial observed in this species is not likely to be a constraint under NT systems, which are known to concentrate weed seeds near the soil surface (Yenish et al. 1996).



Figure 3.23. Effect of seed burial depth on seedling emergence (%) of threehorn bedstraw, small-flowered mallow (scarified seeds) and annual ryegrass. The data of threehorn bedstraw was pooled over the two populations as no interaction was observed between populations and seed burial depth. The data of annual ryegrass was pooled over the years as no interaction was observed between years and depths.

# 3.3.8.3 Annual Ryegrass

Seedling emergence of annual ryegrass was influenced by burial depth (Figure 3.23). The final seedling emergence in the field ranged from 0 to 49%, depending on depth of seed burial. Seedling emergence first increased with shallow burial but then decreased sharply with increased depth of seed burial. Seedling emergence was greatest for seeds buried at 1 cm (49%) followed by seeds buried at 2 cm (44%). Seedling emergence was 16% from the seeds placed on the soil surface. Limited soil-to-seed contact and water availability are some environmental conditions that may limit germination of seeds on the soil surface (Ghorbani et al. 1999). Seedling
emergence was much lower from 5 cm (10%), whereas no seedlings emerged from seeds placed at a depth of 10 cm. The results of this experiment indicate that the farming practices that achieve shallow burial of seeds would promote greater emergence of annual ryegrass seedlings. Seedling emergence from the seeds placed on the soil surface in this experiment was 16% and was similar to the field germination values of 9 to 24% from the seed fate experiment.

## 3.3.8.4 Common Sowthistle

Seedling emergence of common sowthistle decreased with increased burial depth (Figure 3.24). The seedling emergence data of common sowthistle were fitted to an exponential decay curve [E (%) = 77.5 x e<sup>-0.8x</sup>, r<sup>2</sup> = 0.989; where *E* represents seedling emergence (%) and *x* represents depth (cm) of seed burial]. Seedling emergence was the greatest (77%) for seeds placed on the soil surface, and no seedlings emerged from seeds placed at a depth of 5 cm.

The results of this experiment on common sowthistle are consistent with the stimulation of germination in the presence of light. Seeds buried more than 2 mm below the soil surface usually receive less than 1% of incident light (Egley 1986; Woolley and Stoller 1978). Therefore, germination of positively photoblastic seeds is likely to be inhibited even by shallow burial (> 2 mm). In our study, seedling emergence on the soil surface was slightly lower than germination observed in Petri dishes in the light. This difference could be due to less soil-to-seed contact and water availability on the soil surface than on the filter papers (Ghorbani et al. 1999). In an earlier study, Sheldon (1974) obtained 77% emergence from common sowthistle seeds scattered on the soil surface and burial to a depth of 3 cm reduced emergence to 5%. The greater seedling emergence from the seeds placed on the soil surface suggests that under field conditions, NT farming practices would enhance common sowthistle seedling emergence. In such practices, a large proportion of the seed bank remains on the soil surface after crop planting.



Figure 3.24. Effect of seed burial depth on seedling emergence (%) of common sowthistle and wild turnip in a growth chamber at 20/12 C with a 12 h photoperiod. An exponential decay curve was fitted to the common sowthistle data.

#### 3.3.8.5 Wild Turnip

Seedling emergence of wild turnip was influenced by seed burial depth (Figure 3.24). Seedling emergence was increased by shallow burial but then decreased sharply with increased depth of seed burial. Seedling emergence was lower for seeds placed on the soil surface (29%) than for seeds buried at 1 cm (51%) and 2 cm (44%). No seedlings emerged from seeds buried at a depth of 5 cm. Although the cause of low or no germination from deep buried seed was not investigated, it could be due to fatal germination.

In our study, maximum seedling emergence of wild turnip was favoured by shallow burial which is consistent with the results reported by Thanos et al. (1991) for this species. Lower emergence of seeds on the soil surface was probably due to discontinuous or lower availability of moisture, which is supported by the study of osmotic stress where germination in the light/dark regime decreased even at an osmotic potential of -0.1 MPa (Figure 3.16). Light inhibition at this temperature (20/12 C) is not likely to be a major factor (Figure 3.24). However, under suboptimal temperatures in the field, photo-inhibition may have some inhibitory effect on seed germination in wild turnip.

#### 3.3.8.6 Indian Hedge Mustard

The effect of four seed burial depths (0, 2.5, 5 and 10 mm) on seedling emergence of Indian hedge mustard was studied in 2005 and 2006. Seedling emergence was the greatest (70%) for seeds placed on the soil surface, but decreased sharply with increased burial depth (Figure 3.25). No seedlings emerged from seeds buried at a depth of 10 mm. The results of this experiment are consistent with the stimulation of germination in the presence of light. As mentioned earlier, seeds buried more than 2 mm below the soil surface usually receive very less incident light (Egley 1986; Woolley and Stoller 1978). In our study, less than 2% seedling emerged from a depth of 2.5 mm. However in the light/dark experiment, up to 21% seeds germinated in dark. The observed difference between germination in the dark and seedling emergence could be related to small seed size (0.04 mg) of this species, which may make it difficult to emerge even from a burial depth of 2.5 mm.



**Figure 3.25.** Effect of seed burial depth on seedling emergence (%) of Indian hedge mustard in a growth chamber at 25/15 C with a 12 h photoperiod. The data was pooled over the two years as no interaction was observed between year and treatment.

In contrast to Indian hedge mustard, seedling emergence of London rocket was lower for seeds placed on the soil surface than seeds buried at shallow depths (Ray et al. 2005). However, the optimum depth of seedling emergence was only 2 mm. The greater seedling emergence of Indian hedge mustard from the seeds placed on the soil surface suggests that under field conditions, NT farming practices would enhance Indian hedge mustard seedling emergence. In such practices, a large proportion of the seed bank remains on the soil surface after crop planting, and would favour germination and emergence of this positively photoblastic species.

Seedling emergence is known to be influenced by many factors, including weather conditions and soil characteristics (Benvenuti and Macchia 1997). Decreased seedling emergence due to increased burial depth has been reported in several weed species (Benvenuti et al. 2001) which could be linked to seed energy reserves (Mennan and Ngouajio 2006). A possible correlation between seed weight and burial depth required to completely inhibit seedling emergence was explored (Figure 3.26). Smaller seeds tend to germinate better from the soil surface and these weeds may become more problematic in low disturbance cropping systems. Larger seeded weeds, tended to germinate better with shallow burial due to better seed-soil contact. Deep burial inhibited emergence of all weed species, but more so for the smaller seeds that had lower seed reserves (Figure 3.26).



**Figure 3.26.** Relationship between burial depth required to completely inhibit seedling emergence of different weed species and their seed weight.

# 3.3.9 Effect of Tillage Systems on Seedling Emergence Patterns

## 3.3.9.1 Threehorn Bedstraw

A three-parameter sigmoid model {E (%) = $E_{max}/[1+exp^{-(x-t50/Erate)}]$ } was fitted to the seedling emergence (%) of threehorn bedstraw measured at different times after crop sowing (Figure 3.27). Tillage treatment influenced seedling emergence of both

populations of threehorn bedstraw. In both populations, plots sown with MINT had significantly greater seedling emergence than those sown with NT. The maximum seedling emergence of the RC and YP population was 25% and 27% in MINT sown plots as compared to 15% and 18% in NT sown plots. Not only was seedling emergence greater under MINT, the time taken for 50% seedling emergence ( $t_{50}$ ) was also shorter by 2 to 5 days under MINT. Comparisons between the two populations showed that the maximum seedling emergence attained in the field was similar.



Figure 3.27. Effect of minimum tillage (MINT, solid line) and no-till (NT, dotted systems line) on seedling emergence pattern of RC (a) and YP (b) populations of threehorn bedstraw. Vertical bars represent standard errors. three-parameter sigmoid Α model was fitted to the data. Treatments were significantly different (P = 0.05) at all census dates except 7 days after sowing.

Although this study was conducted in only one growing season, the results from the two populations confirmed that seedling emergence in threehorn bedstraw is stimulated by tillage. Within the NT plots, seedling emergence tended to occur within or very near to the crop rows, suggesting that the seedling emergence was promoted by the soil coverage caused by the sowing operation. This is consistent with the inhibitory effects of light on germination reported earlier in this chapter (see section 3.3.1.1). It is likely that a greater proportion of seeds might be left on the soil surface under NT, as minimal soil disturbance occurs. In addition, the increased

burial due to tillage could be linked to better seed-soil moisture contact (and consequently better seed imbibition) of this relatively large seeded species. Germination of a closely related species, catchweed bedstraw, has also been reported to be influenced by cultivation. Froud-Williams (1985) reported that 44% of catchweed bedstraw seedlings emerged following tine cultivation compared to 29% following direct drilling. Similarly, the seedling emergence of both catchweed bedstraw and false cleavers was found to be stimulated by tillage in Canada (Reid and Van Acker 2005).

#### 3.3.9.2 Indian Hedge Mustard

A three-parameter logistic model {E (%) =  $E_{max}/[1 + (x/x_{50})^{E_{rate}}]$ } was fitted to the seedling emergence (%) of Indian hedge mustard measured at different times after crop sowing (Figure 3.28). The seedling emergence of both populations (AFS and E5) of Indian hedge mustard was influenced (P = 0.05) by the tillage systems. In both populations, plots sown with NT had significantly greater seedling emergence than those sown with MINT. The maximum seedling emergence of the AFS and E5 populations ( $E_{max}$ ) was 21% and 18% in NT sown plots as compared to 4% and 3% in MINT sown plots, respectively. The data shows that the maximum seedling emergence attained in the field was similar between the two populations. Regardless of the population, the time taken ( $x_{50}$ ) to achieve 50% of the maximum emergence was lower in the NT system (19 days) than in the MINT system (31 to 32 days). Therefore, NT not only increased  $E_{max}$ , but also reduced  $x_{50}$  by 12 days.

The consistency of results from the two populations confirmed that seedling emergence in Indian hedge mustard is promoted by NT systems. In these systems, most of the seeds are present on the soil surface after crop planting, where light may stimulate seed germination and seedling establishment. In contrast, increased seed burial due to tillage may make it difficult to emerge from greater depths. This is consistent with the results of seed burial experiment, in which seedlings of this species did not emerge even from a depth of 10 mm (Figure 3.25).



Figure 3.28. Effect of minimum tillage (MINT, solid line) and no-till (NT, dotted line) systems on seedling emergence pattern of two populations (AFS and E5) of Indian hedge mustard. Vertical bars represent standard errors. Lines represent the functional three-parameter logistic model fitted to the data. Treatments were significantly different (P =0.05) at all census dates.

# **3.4 Conclusions**

Seed germination of common sowthistle and Indian hedge mustard was stimulated by light. Seedling emergence of these weed species was greatest for the seeds present on the soil surface. These results suggest that common sowthistle and Indian hedge mustard have the potential to become more problematic weeds under NT systems than where tillage occurs. In contrast, light inhibited seed germination in threehorn bedstraw and wild turnip, the latter only at sub-optimal temperatures. Seedling emergence of threehorn bedstraw, wild turnip, small-flowered mallow and annual ryegrass was optimal at shallow depths, which suggests that the farming practices that achieve shallow burial of seeds may promote greater seedling emergence of these weed species.

Seed decay was an important contributor to the loss of seeds of annual ryegrass and common sowthistle from the seed bank in the field and was much greater for seeds after-ripened on the soil surface compared with buried seed. This has implications for annual ryegrass and common sowthistle control in NT farming systems. These systems maintain a large proportion of seeds on the soil surface and will therefore promote quicker depletion of the seed bank. Given that the trend towards reduced tillage systems is likely to continue, weed species such as Indian hedge mustard and common sowthistle may become worse in these systems. However, due to greater seed decay on the soil surface, common sowthistle in addition to annual ryegrass may become easier to manage in NT systems compared to the conventional tillage systems.

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# Chapter 4. Seedling emergence pattern and depth of emergence of several weed species in minimum tillage and no-till systems<sup>\*</sup>

# 4.1 Abstract

Differences in periodicity and depth of weed seedling emergence due to agronomic management practices, such as reduced tillage, have implications for weed competitive ability and management strategies. Periodicity and depth of seedling emergence of 10 different weed species was measured in the field in 2004 and 2005. The seedling emergence of annual ryegrass, threehorn bedstraw and wild radish was greater under minimum tillage than no-till system. In contrast, the seedling emergence of Indian hedge mustard, common sowthistle, silvergrass, small-flowered mallow and turnipweed was greater under the no-till system. The seedling emergence of wild oat and wild turnip was not influenced by the tillage system. The mean seedling emergence depth of wild oat, annual ryegrass, threehorn bedstraw, wild radish and turnipweed was greater under minimum tillage than under the no-till system. These weeds are able to emerge from deeper in the soil profile. In contrast, the seedling emergence depth under minimum tillage and no-till systems was similar for wild turnip, Indian hedge mustard, common sowthistle, small-flowered mallow and silvergrass. These are all small-seeded species, which failed to emerge from deeper depths under either tillage system. In addition, all of these species except wild turnip showed greater total seedling emergence under the no-till system. Results of this study will facilitate weed control timing decisions and provide validation data for weed seedling emergence models.

<sup>\*</sup> The contents of this chapter have been published in 'Weed Science' journal.

Chauhan, B.S., Gill, G. and Preston, C. 2006. Seedling recruitment pattern and depth of recruitment of 10 weed species in minimum tillage and no-till seeding systems. Weed Science 54: 658-668.

# **4.2 Introduction**

Reduced tillage systems have been widely adopted by grain growers in Australia over the past decade to reduce soil erosion, improve soil physical and chemical properties, conserve soil moisture and save on fuel costs. In 2001, more than 40% of the Australian crop was sown with no-till (NT) systems and over the next few years, large increases in the area under NT are expected (D'Emden and Llewellyn 2004). In addition to the soil and agronomic benefits, changes in tillage practices can also have a major influence on the relative abundance of weed species in the field (Froud-Williams et al. 1981).

Weed seedling emergence can be influenced by many factors including tillage (Buhler et al. 1997; Buhler and Mester 1991; Mohler 1993), climate (Baskin and Baskin 1989; Blackshaw et al. 1981) and soil properties. Tillage systems have been shown to affect the vertical seed distribution in the soil (Pareja et al. 1985; Yenish et al. 1992, 1996) and this differential distribution of the seed in the soil profile has the potential to change weed population dynamics (Buhler 1991; Harper 1957). Cultivation affects weed seed germination in the field mainly due to its influence on seed exposure to light (Scopel et al. 1994) or by changing the physical and chemical environment of the seeds. Seeds that germinate too deeply in the soil may fail to emerge.

Generally, the response of weed seedling emergence to tillage is species specific and is dependent on the timing of cultivation relative to the rainfall event (Buhler and Daniel 1988; Ogg Jr. and Dawson 1984; Roberts and Potter 1980). Such interactions between tillage and weed species have the potential to cause major changes in the composition of weed flora in the field with changing tillage systems (Froud-Williams et al. 1981). There has been considerable conjecture in Australia about the effect of NT on the relative abundance of weed species but there is little research data available on this issue. Pratley (1995) reported greater density of fumitory (*Fumaria* spp.) where pre-sowing tillage was given. In an earlier study, Forcella (1984) reported greater abundance of silvergrass (*Vulpia* spp.) in direct drilled crops.

As suggested by Cousens and Moss (1990), burial of weed seeds to variable depths may not only influence total seedling emergence from the seed bank, it could also affect the vigour of the seedlings that establish. This in turn has the potential to influence competitive interactions between crops and weeds. Given that the trend towards NT is likely to continue, research is needed to understand the effects of tillage on shifts in weed flora. Therefore, the major aim of the research reported here was to determine the influence of minimum tillage and NT systems on the seedling emergence pattern and emergence depth of major Australian weed species.

# **4.3 Materials and Methods**

## 4.3.1 Site Description and Source of Seed

Field studies were conducted during the growing season of 2004 and 2005 on the Roseworthy Campus Farm (latitude 34°53'S, longitude 138°69'E) of the University of Adelaide, South Australia. The tillage systems used were NT and minimum-tillage (MINT), which received two pre-sowing cultivations. The characteristics of soil during the duration of experiments are presented in Table 4.1. A range of grass and broadleaf weed species were studied in 2004 and 2005 (Table 4.2).

Year	Texture	pH	OC	TOM
			9	ю́
2004	Heavy clay	7.8	1.99	3.37
2005	Clay loam	7.5	1.49	3.06

**Table 4.1.** Soil characteristics during the duration of experiments<sup>a</sup>.

<sup>a</sup>Abbreviations: OC, organic carbon; TOM, total organic matter

Natural dispersing units (seeds or pods or capsules) of all species except wild turnip (*Brassica tournefortii* Gouan) were collected locally at maturity during November through December of the previous year. Wild turnip pods were collected from Eyre Peninsula, South Australia. Small-flowered mallow (*Malva parviflora* L.) capsules and turnipweed [*Rapistrum rugosum* (L.) All.] pods were collected from the roadside or fence lines, and wild radish (*Raphanus raphanistrum* L.) pods were collected from wheat

(*Triticum aestivum* L.) fields. Wild oat (*Avena fatua* L.), threehorn bedstraw (*Galium tricornutum* Dandy), annual ryegrass (*Lolium rigidum* Gaud.), common sowthistle (*Sonchus oleraceus* L.), and silvergrass (*Vulpia bromoides* (L.) S.F. Gray) seeds were separated from chaff manually. The pods of wild turnip and Indian hedge mustard (*Sisymbrium orientale* L.) were threshed and seeds or pods separated from chaff manually. Seeds of small-flowered mallow were used after capsules were broken to release seeds. Wild radish pods were broken into individual segments and these segments (natural dispersal units) were used as a surrogate for seeds. Similarly, the pods rather than naked seeds of turnipweed were used in the experiments. Hereafter, all natural dispersing units will be referred to as seeds. Wild turnip, small-flowered mallow and turnipweed were only studied in 2005. The size of seeds of different species is presented in Table 4.2.

Weed Species	1000-see	ed weight
	2004	2005
	{	g
Wild oat	22.185	24.425
Wild turnip	NR	0.992
Threehorn bedstraw	5.480	5.279
Annual ryegrass	2.434	2.929
Small-flowered mallow	NR	3.460
Wild radish <sup>b</sup>	4.757	4.615
Turnipweed <sup>c</sup>	NR	0.700
Indian hedge mustard	0.042	0.042
Common sowthistle	NR	0.323
Silvergrass	0.512	0.501

Table 4.2. Weed species and their seed weight<sup>a</sup>.

<sup>a</sup>Abbreviations: NR, not recorded.

<sup>b</sup>Weight of the 1000-dispersal units (segments) was 18.872 and 16.483 g in 2004 and 2005, respectively.

<sup>c</sup>Weight of the 1000-dispersal units (pods) was 5.068 g in 2005.

A known number of weed seeds were spread, 3 to 5 months before the tillage operations, under different tillage treatments (Table 4.3). Seeds were spread in subplots (1 m by 1 m) and seedling emergence was recorded in the whole subplot. Control plots (where weed seeds were not applied) were also established in both years, and seedling emergence was measured in these plots to determine the background seed bank. There was no seedling emergence in the control plots indicating an absence of background seed bank of the species investigated.

**Table 4.3.** Dates of different operations conducted in 2004 and 2005. Weed seeds were spread before cultivation or crop sowing. Two cultivations were given to minimum tillage plots and no cultivation was given to no-till plots.

Operation	2004	2005
Seed/pod/segment numbers	100/m²	200/m <sup>2</sup>
Weed seed spread	13 <sup>th</sup> January	15 <sup>th</sup> March
First cultivation	11 <sup>th</sup> May	03 <sup>rd</sup> May
Second cultivation	18 <sup>th</sup> May	10 <sup>th</sup> June
Crop sowing	06 <sup>th</sup> June	17 <sup>th</sup> June

Two cultivations were given to the MINT plots to a depth of 5 to 8 cm before crop sowing. A field cultivator with narrow shanks (16-mm) and 100-mm wide sweeps was used for cultivation. In NT plots, soil disturbance was limited to the sowing operation only. Wheat cv. Krichauff was sown with a seeder fitted with knife-point soil openers (16-mm wide) in both tillage systems in 2004. In 2005, wide tines (100-mm wide sweeps at the tip of the 16-mm wide shank) were used for sowing the MINT plots to reflect farmer practice. The distance between the crop rows was 25 cm. The experiments were arranged in a split-plot design with tillage systems as the main plots and weed species as the subplots. There were three replicates of each treatment.

#### **4.3.2 Seedling Emergence Patterns and Depth of Emergence**

Weed seedling emergence was measured at different times (days after crop sowing, DAS) and expressed as a percentage of the seed bank of the different species at

sowing. In 2004, depending on the species, seedling emergence was measured at weekly interval for 35 to 56 DAS. The last seedling census was measured at 70 DAS. In 2005, seedling emergence was measured weekly for all species until 63 DAS. The census of seedlings was discontinued when no further emergence was recorded. The weed seedling emergence data are presented against time (DAS) rather than growing-degree days because cumulative growing-degree days were similar between the 2004 (783) and 2005 season (775).

In 2004, seedling emergence depths of two grass weeds (wild oat and annual ryegrass) were measured, while in 2005 seedling emergence depths of all species were measured. Depths of seedling emergence for grass weeds were measured from the seed coat to the soil surface. For broadleaf species, a marker was required to determine the point of germination of the seed (du Croix Sissons et al. 2000). To obtain this, seeds were placed at specific depths in pots and roots were examined at the known planting depth to identify a reliable marker (darkening of the root or sharp change in diameter at the junction of root and shoot) that indicated the position of the seed. The marker for some species was only obvious on young seedlings. Therefore, the plants were removed from the field at an early stage and their roots were cleaned of soil. Care was taken not to disturb the soil surrounding the seedlings. Ten plants per replication were taken unless fewer weeds were present in the plot. Seedling emergence depth of these plants was measured.

#### 4.3.3 Statistical Analyses

Seedling emergence under MINT and NT systems at each sampling time (DAS) was analysed by using analysis of variance (ANOVA). Seedling emergence values for each species under MINT and NT were fitted to a functional three-parameter sigmoid model with the use of SigmaPlot  $9.0^1$ . The model fitted was

$$E(\%) = E_{max} / [1 + exp^{-(x-t50/Erate)}]$$
 [4.1]

where *E* is the total seedling emergence (%) at time *x*,  $E_{max}$  is the maximum seedling emergence (%),  $t_{50}$  is the time to reach 50% of maximum seedling emergence (days)

<sup>&</sup>lt;sup>1</sup>SigmaPlot 2004 (version 9.0), Systat Software, Inc., 501 Canal Boulevard, Suite C, Point Richmond, CA 94804-2028.

and  $E_{rate}$  indicates the slope around  $t_{50}$ . Parameter  $E_{rate}$  provides an indication of the rate of seedling emergence. Parameter estimates were compared by using a two-tailed *t* test (P = 0.05). Seedling emergence depth data for each species was analysed by using ANOVA. Box plots of seedling emergence depth were created using SigmaPlot 9.0. Genstat version 6.0 was used for statistical analysis of data (Genstat 5 Committee 1993).

# 4.4 Results and Discussion

## 4.4.1 Seedling Emergence Patterns and Depth of Grass Weeds

# 4.4.1.1 Annual Ryegrass

Seedling emergence of annual ryegrass was found to be significantly greater (P = 0.05) under MINT at all dates of census in both years (Figure 4.1). NT, which is known to cause lower soil disturbance, had lower maximum seedling emergence (11 to 17%) than MINT (28 to 30%) in both seasons (Table 4.4). Lower seedling establishment under NT could be due to more rapid desiccation of seeds on the soil surface or to greater predation activity of insects on or near the soil surface (e.g. Mohler and Galford 1997). Cultivation has a major influence on the vertical seed distribution in arable soils (Cousens and Moss 1990; Roberts 1963; Staricka et al. 1990) and this can be a critical factor affecting seed survival, germination, and seedling emergence (Mohler 1993).

The start of annual ryegrass seedling emergence (lag phase) was delayed under NT compared with MINT in both years. The time taken for 50% seedling emergence ( $t_{50}$ ) was 3 to 4 days longer under NT. However, once seedling emergence started, the rate (slope  $E_{rate}$ ) was similar between MINT and NT (Table 4.4).

Slower seedling emergence of annual ryegrass under NT could have implications for its management in the field. It could mean that pre-sowing control practices such as non-selective and short residual selective herbicides such as trifluralin, may be less effective under NT than MINT. It could also mean that late emerging annual ryegrass may be less competitive with the crop and not have a substantial impact on crop yield loss and weed seed production (O'Donovan et al. 1985). The difference in seedling emergence between MINT and NT was even greater in 2005, which could be a result of different soil texture or climatic conditions after sowing. In 2005, the soil was lighter textured near the surface (Table 4.1) which may have caused seeds on the surface under NT to dry out more rapidly than seeds buried and insulated by the soil under MINT.



**Figure 4.1.** Seedling emergence pattern of wild oat, annual ryegrass and silvergrass as influenced by minimum tillage (MINT, •, solid line) and no-till (NT, o, dotted line) in 2004 and 2005. Vertical bars represent standard error. Lines represent three-parameter sigmoid model fitted to the data . Seedling emergence (%) data were plotted against time (days after sowing). Asterisk represents significant difference between tillage systems according to ANOVA (P = 0.05).

**Table 4.4.** Seedling emergence pattern response of grassy weeds in minimum tillage (MINT) and no-till (NT). Parameter estimates are followed by standard error (SE) in parentheses and parameter estimates were compared by using a two-tailed *t* test (P = 0.05). Seedling emergence data were fitted to a three-parameter sigmoid model (see Materials and Methods section).  $E_{max}$  is the maximum seedling emergence (%),  $t_{50}$  is the time to reach 50% of maximum seedling emergence (days) and  $E_{rate}$  indicates the slope around  $t_{50}$ .

Tillage	Parameter estimates (SE)							
system	2004			2005				
	E <sub>max</sub>	Erate	<i>t</i> <sub>50</sub>	$r^2$	E <sub>max</sub>	Erate	<i>t</i> <sub>50</sub>	$r^2$
Annual ryegrass								
MINT	27.88	3.61	13.50	0.999	30.03	5.03	14.19	0.999
	(0.19)	(0.16)	(0.17)		(0.44)	(0.45)	(0.51)	
NT	17.40	3.61	17.08	0.997	10.78	4.04	17.92	0.998
	(0.40)	(0.48)	(0.56)		(0.17)	(0.42)	(0.50)	
P value	< 0.001	NS	< 0.001		< 0.001	NS	< 0.001	
			Si	lvergrass				
MINT	-	-	-	-	3.62	3.67	22.47	0.996
					(0.08)	(0.57)	(0.65)	
NT	8.05	4.89	13.46	0.994	12.46	2.50	18.13	0.999
	(0.33)	(0.89)	(1.04)		(0.05)	(0.07)	(0.10)	
P value	-	-	-		< 0.001	NS	< 0.001	
Wild oat								
MINT	10.85	5.84	13.36	0.990	44.96	6.74	20.77	0.995
	(0.60)	(1.28)	(1.51)		(1.33)	(0.90)	(1.05)	
NT	8.13	4.50	16.41	0.989	32.14	6.65	22.44	0.998
	(0.43)	(1.03)	(1.23)		(0.66)	(0.61)	(0.71)	
P value	< 0.001	NS	< 0.01		< 0.001	NS	NS	

Mean seedling emergence depth was significantly greater for annual ryegrass under MINT in both years (Table 4.5 and 4.6). Mean seedling emergence depths for MINT

(13.6 and 14.7 mm) and NT (4.9 and 5.3 mm) were similar when compared across the two years. The variation in seedling emergence depth within the tillage treatment was considerably greater under MINT than NT (Figure 4.2), which may be a reflection of different vertical seed distribution caused by the two tillage systems.

**Table 4.5.** Mean seedling emergence depth (mm) of weed seedlings under minimum tillage (MINT) and no-till (NT) systems in 2004. Depth is followed by standard error in parentheses. Data were analysed by using ANOVA.

Species	Dej	Level of					
	MINT NT		significance				
mm							
Wild oat	21.4 (0.4)	11.5 (1.6)	P < 0.05				
Annual ryegrass	13.6 (1.4)	4.9 (0.7)	P < 0.05				

**Table 4.6.** Mean seedling emergence depth (mm) of weed seedlings under minimum tillage (MINT) and no-till (NT) systems in 2005. Depth is followed by standard error in parentheses. Data were analysed by using ANOVA.

Species	Dej	Level of	
-	MINT	NT	significance
	m	m	
Wild oat	23.9 (2.4)	13.1 (1.7)	NS
Annual ryegrass	14.7 (0.7)	5.3 (0.5)	P < 0.05
Silvergrass	3.7 (0.7)	3.1 (0.3)	NS
Threehorn bedstraw	37.0 (3.7)	27.5 (1.6)	P = 0.05
Wild radish	17.5 (2.5)	12.4 (0.8)	NS
Indian hedge mustard	3.0 (0.3)	2.7 (0.2)	NS
Common sowthistle	8.3 (0.7)	7.3 (1.0)	NS
Turnipweed	23.3 (2.5)	11.8 (0.2)	P = 0.05
Wild turnip	12.3 (1.5)	12.0 (2.4)	NS
Small-flowered mallow	15.6 (0.5)	11.4 (1.4)	NS



**Figure 4.2.** Influence of minimum tillage (MINT, open box) and no-till (NT, striped box) on the variation of seedling emergence depth of different grassy weed species (wild oat, annual ryegrass and silvergrass) in 2004 and 2005. Box plots in which the bottom and top lines are located at the 25th and 75th percentiles of the distribution, showing range from which 50% of seedling emergence occurred (box) and median depth of seedling emergence (line within box), whiskers below and above the box indicate the 10th and 90th percentiles; and solid circle indicate the 5th and 95th percentile.

## 4.4.1.2 Silvergrass

In contrast to the pattern observed in annual ryegrass, seedling emergence of silvergrass was greater under NT in both years (Figure 4.1). In 2004, total seedling emergence from the seed bank was only 0.33% under MINT as compared to 8% under NT sown-plots. In 2005, the maximum seedling emergence was again greater under NT (12.5%) compared with MINT (3.6%). Not only was the seedling emergence level lower under MINT, the time taken for 50% seedling emergence ( $t_{50}$ ) under this treatment was also 4 days longer than under NT (Table 4.4). Deeper burial of seeds under MINT has been reported previously (Yenish et al. 1996) and may be

responsible for the observed seedling emergence behaviour of this small seeded species (Buhler 1991).

Although mean seedling emergence depth was somewhat greater under MINT (3.7 mm) compared with NT (3.1 mm) in 2005, the difference was not statistically significant (Table 4.6). This result is consistent with the general ecological principle that maximum depth of emergence is related to seed size (e.g. Buhler 1991). Although more seeds of silvergrass would have been buried deep under MINT, they failed to emerge and thus had no impact on mean seedling emergence depth. Seedling emergence of silvergrass has been reported to be severely reduced at depths greater than 10 mm (Dillon and Forcella 1984). Therefore, this weed is not expected to be a major problem under MINT, but adoption of direct-drill techniques has been responsible for its increasing prominence as a weed in cereal crops (Amor and de Jong 1983).

# 4.4.1.3 Wild Oat

Although the seedling emergence of wild oat was somewhat greater under MINT as compared to NT in both years, the difference was not statistically significant (Figure 4.1). In 2004, the maximum seedling emergence estimated from the fitted model (Equation 4.1) was greater under MINT (10.9%) than NT (8.1%). The time taken for 50% of seedlings to emerge ( $t_{50}$ ) was less under MINT (Table 4.4). However, the rate of seedling emergence (slope  $E_{rate}$ ) was very similar between MINT and NT. In 2005, the maximum seedling emergence from the seed bank was much greater than in 2004, with MINT and NT showing average seedling emergence of 45 and 32%, respectively (Table 4.4). Again the slope and  $t_{50}$  were similar between MINT and NT.

Wild oat seeds are known to have the capacity to bury themselves into the soil during repeated hydration/dehydration cycles (Sharma and Vanden Born 1978). Therefore, it is likely that germination and establishment in this species may not be very responsive to being left on the soil surface under NT. However, Miller and Nalewaja (1985) found lower wild oat populations under zero tillage. Although in our study the difference in seedling emergence between MINT and NT system was not significant,

exposure to light can inhibit seedling emergence from the soil surface under NT (Sharma and Vanden Born 1978). The maximum seedling emergence under both systems was greater in 2005 as compared to 2004 (Figure 4.1). This could be due to seasonal effects on the dormancy of this species (Andersson and Milberg 1998).

The depth of seedling emergence of wild oat was measured in both 2004 and 2005. The mean seedling emergence depth was greater under MINT (21.4 to 23.9 mm) than NT (11.5 to 13.1 mm) in both years (Table 4.5 and 4.6); however, the difference was statistically significant only in 2004. Similarly, mean seedling emergence depth for wild oat was found to be significantly shallower under zero tillage than MINT fields (du Croix Sissons et al. 2000). In our study, maximum depths of wild oat seedling emergence were 32 and 46 mm in 2004 and 2005, respectively and occurred in MINT (Figure 4.2). This is not surprising considering wild oat seeds have been shown to emerge from as deep as 200 mm (Sharma and Vanden Born 1978). In our study, however, such depths are well beyond the depth of cultivation and no seed is likely to have ended up that deep.

## 4.4.2 Seedling Emergence Patterns and Emergence Depth of Broadleaf Weeds

## 4.4.2.1 Threehorn Bedstraw

In both years of our study, plots sown with MINT had significantly greater seedling emergence of threehorn bedstraw compared with NT (Figure 4.3). The maximum seedling emergence in 2004 and 2005 was 14 and 26% in MINT-sown plots as compared with 9 and 18% in NT-sown plots (Table 4.7).

The greater seedling emergence under MINT might be due to inhibition of germination in this species by exposure to light. It is plausible that a greater proportion of threehorn bedstraw seeds may be left on the soil surface under NT, as only minimal soil disturbance occurs with the seeding operation. Germination of a closely related species, false cleavers (*Galium spurium* L.), has been reported to be strongly inhibited by light (Malik and Vanden Born 1987). Froud-Williams (1985) reported that 44% seedlings of cleavers (*G. aparine* L.) emerged following tine cultivation as compared to 29% following direct drilling.



**Figure 4.3.** Seedling emergence pattern of threehorn bedstraw, wild radish, common sowthistle and Indian hedge mustard as influenced by minimum tillage (MINT, •, solid line) and no-till (NT, o, dotted line) in 2004 and 2005. Vertical bars represent standard error. A three-parameter sigmoid model was fitted by using SigmaPlot 9.0. Seedling emergence (%) data were plotted against time (days after sowing). Asterisk represents significant difference between tillage systems according to ANOVA (P = 0.05).

**Table 4.7.** Seedling emergence pattern response of broadleaf weeds in minimum tillage (MINT) and no-till (NT). Parameter estimates are followed by standard error (SE) in parentheses and parameter estimates were compared by using a two-tailed t test (P = 0.05). Seedling emergence data were fitted to a three-parameter sigmoid model (see Materials and Methods section).  $E_{max}$  is the maximum seedling emergence (%),  $t_{50}$  is the time to reach 50% of maximum seedling emergence (days) and  $E_{rate}$  indicates the slope around  $t_{50}$ .

Tillage	Parameter estimates (SE)							
system	2004			2005				
	E <sub>max</sub>	$E_{rate}$	<i>t</i> <sub>50</sub>	$r^2$	$E_{max}$	$E_{rate}$	<i>t</i> <sub>50</sub>	$r^2$
Threehorn bedstraw								
MINT	14.18	6.03	24.15	0.998	25.62	2.75	18.45	0.999
	(0.34)	(0.57)	(0.69)		(0.24)	(0.20)	(0.26)	
NT	8.69	4.67	26.20	0.995	17.66	4.89	23.79	0.993
	(0.27)	(0.67)	(0.79)		(0.56)	(0.83)	(0.98)	
P value	< 0.001	NS	NS		< 0.001	< 0.05	< 0.001	
			W	ild radish				
MINT	3.07	8.82	17.56	0.991	10.43	6.32	21.98	0.998
	(0.16)	(1.63)	(1.86)		(0.19)	(0.54)	(0.63)	
NT	1.38	8.10	25.35	0.983	6.25	6.04	31.64	0.999
	(0.10)	(1.81)	(2.30)		(0.11)	(0.41)	(0.50)	
P value	< 0.001	NS	< 0.05		< 0.001	NS	< 0.001	
			Indian	hedge mus	stard			
MINT	7.58	3.31	14.53	0.990	1.66	3.26	21.72	0.996
	(0.34)	(0.90)	(0.97)		(0.34)	(0.56)	(0.60)	
NT	15.47	5.21	12.28	0.994	16.91	2.41	16.25	0.999
	(0.63)	(0.93)	(1.08)		(0.10)	(0.12)	(0.16)	
P value	< 0.001	NS	NS		< 0.001	NS	< 0.001	
Common sowthistle								
MINT	3.99	3.66	22.13	0.990	5.66	3.10	22.19	0.998
	(0.18)	(0.85)	(0.98)		(0.09)	(0.38)	(0.41)	
NT	15.68	3.57	17.80	0.995	13.57	3.35	18.96	0.999
	(0.47)	(0.60)	(0.73)		(0.18)	(0.33)	(0.38)	
P value	< 0.001	NS	< 0.01		< 0.001	NS	< 0.001	

When the two growing seasons were compared, total seedling emergence under MINT was greater in 2005 (26%) than in 2004 (14%). It is possible that differences in maternal conditions during seed ripening may be responsible for differential expression of dormancy in the locally produced seeds of this species used over the two years of this study (Andersson and Milberg 1998).



**Figure 4.4.** Influence of minimum tillage (MINT, open box) and no-till (NT, striped box) on the variation of seedling emergence depth of different broadleaf weed species (threehorn bedstraw, wild radish, turnipweed, Indian hedge mustard, wild turnip, common sowthistle and small-flowered mallow) in 2005. Box plots in which the bottom and top lines are located at the 25th and 75th percentiles of the distribution, showing range from which 50% of seedling emergence occurred (box) and median depth of seedling emergence (line within box), whiskers below and above the box indicate the 10th and 90th percentiles; and solid circle indicate the 5th and 95th percentile.

Seedling emergence depth of threehorn bedstraw was deeper and more variable in MINT than NT (Table 4.6; Figure 4.4). Mean seedling emergence depth was 37 mm under MINT and 27.5 mm under NT. The deeper burial of threehorn bedstraw seeds may have been responsible for greater time taken to reach  $t_{50}$  under MINT than NT (Table 4.7). Inhibition of germination in this species in surface seed by light

exposure would have tended to increase the mean depth of seedling emergence, particularly in NT.

## 4.4.2.2 Wild Radish

The seedling emergence pattern of wild radish was significantly (P = 0.05) influenced by tillage system in both years (Figure 4.3). MINT had greater seedling emergence than NT in both years; however, the difference was not significant until 21 DAS in 2004 and 14 DAS in 2005. Maximum seedling emergence at the date of last seedling census (70 DAS) in 2004 was only 3.1 and 1.4% under MINT and NT, respectively (Table 4.7). In 2005, the maximum seedling emergence was 10.4 and 6.3% under MINT and NT.

Cultivation can promote seedling emergence in wild radish because germination in this species is stimulated by darkness (Piggin et al. 1978). In a later study Reeves et al. (1981) also showed strong stimulation of seedling emergence from wild radish seed bank by cultivation. As seen in the case of wild oat and threehorn bedstraw, maximum seedling emergence in 2005 was much greater than that in 2004 and might be due to differences in the dormancy status of the seed between seasons. Pod characteristics play an important role in the expression of dormancy in wild radish (Cheam 1986; Mekenian and Willemsen 1975). The weight (size) of the pod segments was smaller in 2005 than 2004 (Table 4.2), which could be related to a thinner pod wall in that season. Greater seedling emergence in wild radish in 2005 may also be due to the seasonal effects on the percentage of hard seeds present.

As shown by the differences in  $t_{50}$  between the tillage treatments (Table 4.7), wild radish seedling emergence was delayed by 7 to 9 days under NT than MINT. This is likely due to stimulation of germination in buried seeds (dark) under MINT. Plants emerging earlier tend to be more vigorous and competitive and can produce greater number of seeds than those emerging later. They can also produce a greater proportion of dormant seeds (e.g. Cheam 1986; Fernandez-Quintanilla et al. 1986; Mack and Pyke 1983; Mohler and Calloway 1995). There was a considerable overlap in the range of seedling emergence depths under MINT and NT (Figure 4.4). However, as in the case of most other species, median emergence depth under MINT was nearly double that recorded under NT. This difference in seedling emergence depth between MINT and NT appears to be simply a reflection of vertical seed distribution caused by the two tillage systems.

### 4.4.2.3 Indian Hedge Mustard

The seedling emergence of Indian hedge mustard was significantly greater (P = 0.05) under NT as compared to MINT in both years (Figure 4.3). The differences between the two tillage systems were particularly large in 2005, when maximum seedling emergence ( $E_{max}$ ) under NT was 17% as compared to 1.7% under MINT (Table 4.7). Greater seedling emergence of this species under NT is likely related to its small seed size (Table 4.2), which failed to emerge when buried deeply by MINT. In both years, NT promoted earlier seedling emergence compared with MINT, and the time taken for 50% seedling emergence ( $t_{50}$ ) was 2 to 5 days shorter under NT than MINT (Table 4.7).

The difference in seedling emergence between MINT and NT was greater in 2005 than 2004 and may be related to deeper burial of seeds by the wider tines used for sowing operation tillage in 2005. Moreover, dormancy might have been induced in this light-requiring species by burial under MINT (Pons 1989; Wesson and Wareing 1969). Previous studies have shown that less than 1% of incident light can penetrate further than 2.2 mm into soil (Egley 1986; Woolley and Stoller 1978). Therefore, seeds of Indian hedge mustard buried deep by tillage are likely to remain dormant at least in the short term and fail to contribute to seedling emergence.

The depth of seedling emergence was similar under MINT (3.0 mm) and NT (2.7 mm; Table 4.6). Because Indian hedge mustard has a small seed and requires light exposure for germination to occur (Pons 1989), this result is not surprising. Greater seedling emergence in Indian hedge mustard under NT in this study indicates that this species has the potential to become a problematic weed under reduced tillage systems.

## 4.4.2.4 Common Sowthistle

The seedling emergence of common sowthistle was found to be significantly greater under NT than MINT in both years of this study (Figure 4.3). The maximum seedling emergence ( $E_{max}$ ) over the two years ranged from 4.0 to 5.7% under MINT and 13.6 to 15.7% under NT (Table 4.7). The time taken for 50% of seedlings to emerge ( $t_{50}$ ) was lower in NT as compared to MINT. Taken together, these results indicate greater and faster seedling emergence in this species under NT than MINT. The small size of common sowthistle seeds (Table 4.2) would render seedling emergence difficult from greater depths in MINT.

Previous research has shown greater abundance of wind-dispersed species under reduced tillage, which might be due to lack of burial by tillage equipment (Derksen et al. 1993; Froud-Williams et al. 1981). Recently, Widderick et al. (2004) reported that seedling emergence of common sowthistle was much greater under zero tillage systems than more aggressive tillage systems.

As in the case of Indian hedge mustard, the depth of seedling emergence was shallow but similar under both MINT (8.3 mm) and NT (7.3 mm; Table 4.6). Even though a greater proportion of common sowthistle seeds would have been buried deep under MINT, they would have failed to establish and therefore had no impact on the mean seedling emergence depth. As common sowthistle showed a distinct preference for NT for seedling emergence, this species is expected to be more problematic under reduced tillage systems. This conclusion is supported by the recent field assessments of Widderick et al. (2004) in southern Queensland.

## 4.4.2.5 Wild Turnip

The influence of tillage on the seedling emergence of wild turnip was studied only in 2005. Although there was a trend for greater seedling emergence under MINT than NT, the differences were not significant (P > 0.05; Figure 4.5). The maximum seedling emergence under MINT and NT was 3.2 and 0.7%, respectively (Table 4.8). There are reports of inhibition of germination in this species by light (Cousens et al. 1993). Therefore, exposure to light of seeds on or near soil surface may have inhibited seedling emergence under NT. However, the stimulatory effect of tillage

might have been dampened by the failure of seedlings to emerge from depth under MINT. Being a small-seeded species (Table 4.2), it might be difficult for this species to emerge when germination takes place deep in the soil. This is supported by the data on the depth of seedling emergence, which was very similar under MINT (12.3 mm) and NT (12 mm; Table 4.6).



Figure 4.5. Seedling of wild emergence pattern turnip, small-flowered mallow and turnipweed as influenced by minimum tillage (MINT, •, solid line) and no-till (NT, o, dotted line) in 2005. Vertical bars represent standard error. A three-parameter sigmoid model was fitted by using SigmaPlot 9.0. Seedling emergence (%) data were plotted against time (days after sowing). Asterisk represents significant difference between tillage systems according to ANOVA (P = 0.05).

# 4.4.2.6 Small-Flowered Mallow

The seedling emergence of small-flowered mallow was significantly lower under MINT than NT at all dates of census (Figure 4.5). Most of the seedlings of small-

flowered mallow emerged within 14 days of crop sowing. Although emergence in NT was only 50% greater than MINT, this study provides some support for field observations that densities of small-flowered mallow were greater under NT. The time taken for 50% of seedlings to emerge was 2 days lower under NT than MINT. Although there was a trend for greater seedling emergence depth under MINT (15.6 mm) than NT (11.4 mm), the difference was not significant (Figure 4.4; Table 4.6).

The seed of this species has a hard seed coat which needs to be scarified to stimulate germination (Chorbadjian and Kogan 2004). As most of the seeds stay on the soil surface under NT, they would experience greater fluctuation in temperature and moisture regime, which might help in scarifying the seed coat (e.g. reviewed by Taylor 2005). In contrast, Buhler and Daniel (1988) reported that termination of velvetleaf (*Abutilon theophrasti* L.) dormancy, also a species with hard seed coat, was more effective under conventional tillage due to naturally occurring fractures possibly caused by scarification of seed coats by soil-particle movement during tillage.

## 4.4.2.7 Turnipweed

Final seedling emergence of turnipweed was more than threefold greater (P = 0.05) under NT than MINT (Figure 4.5; Table 4.8), however, the differences between tillage systems were not significant until 21 DAS. Although this species appears to have inherently low levels of seedling emergence from the seed bank, the maximum seedling emergence under MINT was much lower (1.8%) than that recorded under NT (5.8%). The time taken for 50% of seedlings to emerge ( $t_{50}$ ) was greater under NT than MINT (Table 4.8).

The greater level of seedling emergence under NT relative to MINT could be due to greater breakdown of pods by the environmental conditions on the soil surface. The results of this study are consistent with the observation of dominance of this species in undisturbed habitats such as along fence lines and roadsides.
Not surprisingly, the mean seedling emergence depth of turnipweed was significantly greater under MINT than NT (Table 4.6) and depth of seedling emergence was also much more variable under MINT than NT (Figure 4.4).

**Table 4.8.** Seedling emergence pattern response of broadleaf weeds in minimum tillage (MINT) and no-till (NT) in 2005. Parameter estimates are followed by standard error (SE) in parentheses and parameter estimates were compared by using a two-tailed *t* test (P = 0.05). Seedling emergence data were fitted to a three-parameter sigmoid model (see Materials and Methods section).  $E_{max}$  is the maximum seedling emergence (%),  $t_{50}$  is the time to reach 50% of maximum seedling emergence (days) and  $E_{rate}$  indicates the slope around  $t_{50}$ .

Tillage system	Parameter estimates (SE)					
	E <sub>max</sub>	$E_{rate}$	$t_{50}$	$r^2$		
	Wild turnip					
MINT	3.17	3.71	19.10	0.999		
	(0.03)	(0.20)	(0.24)			
NT	0.68	5.19	19.14	0.987		
	(0.03)	(1.27)	(1.47)			
<i>P</i> value	< 0.001	NS	NS			
		Small-flowe	red mallow			
MINT	6.11	4.48	8.07	0.991		
	(0.21)	(1.12)	(1.22)			
NT	7.99	2.21	5.81	0.993		
	(0.23)	(0.85)	(0.78)			
<i>P</i> value	< 0.001	NS	NS			
	Turnipweed					
MINT	1.80	2.27	21.42	0.998		
	(0.03)	(0.44)	(0.33)			
NT	5.80	7.70	24.62	0.996		
	(0.18)	(0.89)	(1.06)			
<i>P</i> value	< 0.001	NS	< 0.05			

# **4.5 Conclusions**

The influence of tillage systems on weed seedling emergence pattern could be due to tillage effects on the vertical seed distribution in the soil. Tillage affects vertical seed placement zone, which in turn affects weed seedling emergence pattern and weed population. In addition to vertical seed distribution, tillage systems also affect the soil condition surrounding the seed and these both effects can interact to affect seedling emergence. Small-seeded species mainly emerge from the surface layer; consequently these species have a greater potential to emerge effectively under NT systems. Some small-seeded species also require light for germination. In contrast, large-seeded weed species were favoured by MINT. These species have lower dependency on light for germination as well as greater energy reserves for deeper emergence. In addition, those seeds that require darkness for germination will also not germinate under NT unless covered by soil during the sowing operation. However, this discussion assumes the effects of rain and wind on movement of seeds are negligible. Weed seedlings emerging faster and earlier than the crop or other weeds have the potential to be more competitive with the crop and to cause greater yield loss (e.g. O'Donovan et al. 1985).

Depth of seedling emergence is dependent on the vertical seed distribution caused by the tillage system, whereas species responses are dependent on seed size and species response to light. Small-seeded species tend not to emerge from depth, probably because they have insufficient reserves to emerge from depth. Large-seeded species, in contrast, are able to emerge from depth. Such weeds tended to emerge from greater mean depth in MINT than in NT. However, seedlings emerging later because of deeper burial are likely to be at a competitive disadvantage against plants of the same or other species that emerged earlier from a shallow depth.

The information on the effect of tillage systems on the seedling emergence of different weed species is useful for developing and/or validating prediction models. Such information can also be useful in deciding optimal timing for the control of weeds in a crop. The study has clearly shown a large inhibition in seedling emergence of annual ryegrass under NT. Such a result raises an important question: what is the long-term fate of the seeds that remain in the seed bank as a consequence

of lower seedling emergence? Do the weed seeds that fail to germinate under NT decay before the start of the next growing season or do they become part of a more persistent seed bank? There is an urgent need for further research to address this key knowledge gap in our understanding of the ecology of agricultural weeds.

The information on the influence of tillage systems on the persistence of annual ryegrass seed bank is presented in the next chapter (Chapter 5).

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# Chapter 5. Influence of tillage systems on vertical distribution, seedling emergence and persistence of annual ryegrass seed bank<sup>\*</sup>

# 5.1 Abstract

Several studies were conducted to evaluate the effect of different tillage systems on the vertical seed distribution, seedling emergence pattern and persistence of annual ryegrass seed bank. Experiments were conducted in South Australia at two locations (Roseworthy Campus and Minlaton) in 2003 and 2005. The distribution of surface seeds through the soil profile was associated with the level of soil disturbance. The low soil disturbance tillage systems left more seeds on the soil surface, whereas the high soil disturbance systems buried most of the seeds. The seedling emergence of annual ryegrass was lower under the low soil disturbance tillage systems than the high soil disturbance tillage systems at both locations. The seedling emergence was two to four-fold greater under minimum tillage than no-till. Not only was the seedling emergence lower under the low soil disturbance tillage systems, biomass accumulation by annual ryegrass seedlings was also lower under these systems. The carryover of residual viable seeds from one season to the next was similar between the tillage systems. However, seed decay under no-till (48 to 60%) was much greater than minimum tillage (12 to 39%).

# **5.2 Introduction**

Over the past decade there has been a rapid increase in the use of reduced tillage for crop production in Australia. In 2001, more than 40% of the cropping area in Australia was under no-till (NT) and over the next few years, this area is expected to

<sup>\*</sup> The contents of this chapter have been published in 'Weed Science' journal.

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increase dramatically (D'Emden and Llewellyn 2004). These changes in tillage practices can have a major influence on weed ecology including seedling emergence patterns of weed species in the field. The level of soil disturbance from pre-sowing tillage and the sowing operation is a major determinant of the vertical distribution of weed seeds in the soil (Pareja et al. 1985; Yenish et al. 1992, 1996), which can have a major impact on weed population dynamics (Buhler 1991). However, the impact of tillage systems on weed seedling emergence under the Mediterranean climatic conditions experienced in southern Australia has not been studied.

The soil disturbance caused by tillage systems places weed seeds at different depths, which differ in availability of moisture, diurnal temperature fluctuation, light exposure and activity of predators. All these microenvironment attributes have the potential to influence the behaviour of weed seed banks. In addition to the effects on the periodicity of seedling emergence, burial of weed seeds to different depths can also affect total seedling emergence from the seed bank, as well as the vigour of the seedlings that establish. Weed seedlings emerging earlier may have more vigour than the late emerging ones, which in turn could influence competitive interactions between crops and weeds (e.g. O'Donovan et al. 1985).

Because of its propensity to rapidly evolve herbicide resistance, annual ryegrass (*Lolium rigidum* Gaud.) is widely considered to be one of the worst weeds of cropping systems (Heap 2004). However, it is unclear how this species responds to changes in tillage systems. Research from elsewhere in the world has demonstrated that weed seedling emergence responses to tillage systems are species specific. In some weed species that possess a hard seed coat, such as velvetleaf (*Abutilon theophrasti* Medik.), seedling emergence from the seed bank was lower under the NT systems compared to the conventional systems (Buhler and Daniel 1988). However, in the same study a grass species (giant foxtail, *Setaria faberi* Herrm.) showed much greater infestation under NT.

In annual ryegrass there is some evidence for lower seedling emergence under untilled compared to tilled systems (Peltzer and Matson 2002). At this stage the longterm fate of seeds that remain in the seed bank as a consequence of lower seedling emergence under NT seeding systems is not known. These seeds may decay before the start of the next growing season or may become part of a more persistent seed bank. The answers to these questions could have significant implications for weed management. Given that the trend towards reduced tillage systems is likely to continue, research is needed to understand the behaviour of the seed bank under changing tillage systems. Such knowledge is particularly important for major weed problems such as annual ryegrass. The aim of this research was to determine the influence of different tillage systems on the vertical distribution, seedling emergence pattern and persistence of the annual ryegrass seed bank.

# **5.3 Materials and Methods**

#### **5.3.1 Site Description**

The experiments were conducted during the growing seasons of 2003 and 2005 at two different sites: at the Roseworthy Campus Farm (latitude 34°53'S, longitude 138°69'E) of the University of Adelaide and at Minlaton (latitude 34°77'S, longitude 137°59'E) on the Yorke Peninsula of South Australia. The climate of both locations is Mediterranean and is characterised by cool wet winters and hot dry summers. At the Roseworthy Campus, the texture of the field soil was heavy clay and clay loam in 2003 and 2005, respectively. At Minlaton, the soil texture was sandy loam and heavy clay in 2003 and 2005, respectively. The climatic data from June to September of each year are presented in Figure 5.1.

#### 5.3.2 Tillage Systems

The tillage systems used at the Roseworthy Campus were minimum tillage (MINT) and NT. MINT plots were cultivated twice to a depth of 8 to 10 cm before crop sowing. The width of the tines used for cultivation was 100-mm. In the NT plots, soil disturbance was limited to the sowing operation only. In 2003, wheat (*Triticum aestivum* L. cv. Krichauff) was sown with a seeder fitted with knife-point soil openers (16-mm wide) in both tillage systems. In 2005, 100-mm wide tines were used for sowing the MINT plots to reflect farmer practice, but the NT plots were sown with knife-point soil openers. The crop was sown on 28th May, 2003 and 17th



June, 2005. The experiments were arranged in a randomised complete block design with three replications.

**Figure 5.1.** Maximum and minimum temperature (C) and rainfall (mm) recorded during June to September in the field during 2003 and 2005 at the Roseworthy Campus and Minlaton site.

The tillage systems at Minlaton comprised of two disc systems, DayBreak  $(DB)^1$  and K-Hart  $(KH)^2$ ; and two tine based systems, narrow point  $(NP)^3$  and Ribbon seeder  $(RS)^4$ . The images of these tillage systems are presented in Figure 5.2. No prior tillage was conducted before wheat sowing at this site. Wheat at Minlaton was sown on 15th June, 2003 and 11th July, 2005. The experiments were arranged in a randomised complete block design. There were four replicates of each treatment in 2003 and three replicates in 2005.

# 5.3.3 Vertical Distribution of Seeds in the Soil

Vertical seed distribution was studied by using either annual ryegrass seeds or plastic beads (2000/m<sup>2</sup>; 1.5 mm by 1.0 mm size; 5 mg wt). At the Roseworthy Campus site in 2003, seeds from the natural seed bank were counted to quantify the vertical seed distribution. This site had been under NT for many years before this experiment, so most of the seeds would have been present on or near the soil surface before the start of the experiment. In 2005, plastic beads were uniformly broadcast in each plot over an area of 0.5 m<sup>2</sup> just before cultivation or sowing. Four soil cores (5 cm diameter) per plot were taken immediately after the sowing operation. The soil cores from each plot were divided into different depths (2003: 0 to 1, 1 to 5 and 5 to 10 cm; 2005: 0 to 2, 2 to 5 and 5 to 10 cm). All samples for a given depth from each plot were combined for analysis. The soil samples were washed to separate the seeds or beads

<sup>&</sup>lt;sup>1</sup>Single-angled disc, double-shoot opener DayBreak, Milne Industries, PO Box 735, Dalby, Queensland 4405, Australia.

<sup>&</sup>lt;sup>2</sup>Rippled-disc fertilizer-banding coulter (Yetter Manufacturing Co., USA) combined with twin seeding disc unit, K-Hart Industries, Canada. Importer in Australia: Direct Seeding and Harvesting Equipment, RMB 94, Wellstead, Western Australia 6328, Australia.

<sup>&</sup>lt;sup>3</sup>Sixteen-millimeter wide knife blade (PR 87) with rubber seed boot double shoot kit attachment (Primary Sales Australia), fitted onto 550 pound-force rated C-shank tine (Flexi-Coil) with 100 mm wide blanked press wheel, Primary Sales Australia, 44 Meliador Way, Midvale, Western Australia 6056, Australia.

<sup>&</sup>lt;sup>4</sup>Anderson A12 double shoot Ribbon seeding opener, fitted on 220 pound-force rated C-shank tine (Flexi-Coil), with single sided rolling shield attachment (soil throw equaliser) and 165 mm wide flat press wheel, Importer in Australia: CNH Global, Locked Bag 3, St Marys, New South Wales 2790, Australia.

from the soil. The seeds or beads were counted for each depth and presented as percent of total seeds or beads present at all depths.

At Minlaton, annual ryegrass seeds  $(2000/m^2)$  coated with a fluorescent pigment (Blaze Orange<sup>®</sup>) were uniformly broadcast in each plot over an area of 0.5 m<sup>2</sup> just before sowing in both years. Four soil cores (5 cm diameter) from different depths (0 to 1, 1 to 5 and 5 to 10 cm) were taken immediately after the sowing operation. Seeds that fluoresced under ultraviolet light in the laboratory darkroom were counted for each depth (Figure 5.3) and presented as percent of total seeds present at all depths.



DayBreak



**K-Hart** 



**Narrow point** 



**Ribbon seeder** 

Figure 5.2. Images of tillage systems used at the Minlaton site.

# 5.3.4 Seedling Emergence Under Different Tillage Systems

At each site, the natural seed bank was studied in 2003, but seed was spread manually in 2005. Seeds were collected from wheat fields (during November to December) and separated from chaff manually. Seeds were stored in a glasshouse

until spread in the experimental plots. At the Roseworthy Campus site in 2005, four populations (RCE1, RCE5, RCE9 and RCNW7) of annual ryegrass were studied. Seeds of all populations (2700 seeds/m<sup>2</sup> for each population) were spread in March 2005. Because all tillage systems at Minlaton were direct-drilled, seeds of a local population (2000 seeds/m<sup>2</sup>) were spread before the sowing of wheat. Two permanent quadrats (0.4 m by 0.4 m) were placed in each plot to determine the seedling emergence pattern under different tillage systems. Annual ryegrass seedlings emerging within these quadrats were counted at different times after wheat was sown.



**Figure 5.3.** Fluoresced seeds of annual ryegrass under ultraviolet light in a dark laboratory room.

Control plots (where weed seeds were not applied) were also established at both sites in 2005 and seedling emergence measured in these plots to determine the background seed bank. However, there was no seedling emergence in the control plots, indicating absence of background seed bank of annual ryegrass in 2005.

In order to determine the vigour of annual ryegrass plants under different tillage systems, 75 plants per tillage treatment were harvested separately (at both sites in 2005), placed in paper bags, and oven-dried at 65 C for 48 hours for dry weight measurements. Plants of only one population, RCE5, were sampled from the Roseworthy Campus site. The plants were sampled at 112 days after sowing (DAS) from the Roseworthy Campus site and 75 DAS from the Minlaton site.

#### 5.3.5 Seed Bank Persistence Under Different Tillage Systems

To estimate the initial seed bank of annual ryegrass in 2003, 20 soil cores (7 cm in diameter) were taken immediately after the wheat was sown. In each plot, cores were taken diagonally across each plot (10 cores per diagonal) and to a depth of 10 cm. The soil samples were washed through metal sieves (0.5 mm diameter) and then dried at 40 C prior to counting the number of annual ryegrass seeds in each sample. To estimate the residual seed bank, 20 soil cores were taken in the spring, prior to new seed-set, from the plots sampled earlier at sowing. These cores were treated by the method described above. Seeds were assessed for viability by slicing longitudinally to expose the embryo, and incubating in 1% (w/v) tetrazolium chloride solution for 24 hours in the dark at 30 C (Steadman et al. 2003). The extent of pink staining was observed through a microscope and when complete staining of the embryo was observed, seeds were scored as viable. Seeds that lacked integrity of embryo and endosperm (black powder) were considered to have decayed.

At Minlaton, soil at the experimental field during the 2003 season was suspected of being non-wetting (water repellent) in nature. Therefore, soils present in all experimental fields were tested to categorise their severity of water repellence by ethanol droplet test described by King (1981). Each treatment was replicated four times. The field soils from both sites had very low level of water repellence, except the field soil from Minlaton in 2003, which had a moderate level.

#### **5.3.6 Statistical Analyses**

Vertical seed distribution under different tillage systems was analysed by using twoway analysis of variance (ANOVA) in a randomised block design with tillage and depth as the two factors. Seedling emergence under different tillage systems at each sampling time (DAS) was analysed by using ANOVA in a randomised block design. Seedling emergence values under different tillage systems were fitted to a functional three-parameter logistic model using SigmaPlot 2004 (version 9.0). The model fitted was:

$$E = E_{max} / [1 + (x/t_{50})^{Erate}]$$
[5.1]

where *E* is the total seedling emergence (plants/m<sup>2</sup>) at time *x*,  $E_{max}$  is the maximum seedling emergence (plants/m<sup>2</sup>),  $t_{50}$  is the time to reach 50% of maximum seedling

emergence (days) and  $E_{rate}$  indicates the slope. Parameter estimates were compared using a two-tailed *t* test (P = 0.05). The different components of seed fate on both sites were analysed by using ANOVA in a randomised block design. ANOVA was used to compare the dry matter of each annual ryegrass plant. All data were tested for homogeneity of variance to determine if transformations were necessary. Genstat version 6.0 was used for statistical analysis of data (Genstat 5 Committee 1993).

# 5.4 Results and Discussion

#### 5.4.1 Vertical Distribution of Seeds in the Soil

At the Roseworthy Campus site in 2003, the vertical distribution of seeds at all depths (0 to 1, 1 to 5, and 5 to 10 cm) was significantly influenced by tillage system (Figure 5.4). The low soil disturbance NT retained 56% of the seeds in the top 1 cm soil layer, whereas the high soil disturbance MINT buried 65% of the seeds to a depth of 1 to 5 cm and only 5% of the seeds remained in the top 1 cm soil layer in this system. Similarly in 2005, NT left more than 70% of the beads, representing seeds, in the top 2 cm soil layer (Figure 5.4). In contrast, MINT buried 67% of the beads to a depth of 2 to 5 cm and only 21% of the beads remained in the top 2 cm soil layer in this system.

At the Minlaton site, the vertical seed distribution was studied under four different direct-drilled disc and tine systems. In 2003, the low soil disturbance DB system retained more than 75% of the annual ryegrass seeds on the soil surface (0 to 1 cm), whereas the high soil disturbance RS buried more than 75% of the seeds to a depth of 1 to 5 cm (Figure 5.5). The RS system left only 11% of seeds on the soil surface while the NP system was intermediate, with 42% of the seeds remaining on the soil surface. Irrespective of the tillage system, only a small proportion of the total seed bank was found in the 5 to 10 cm soil layer.

The trend was similar in 2005, when 85% of the seeds were present in the top 1 cm of the soil layer under DB, whereas KH, NP and RS retained 66, 40 and 14% of the seeds at this depth, respectively (Figure 5.5). RS buried more than 75% of the seeds



to a depth of 1 to 5 cm, whereas NP, KH and DB retained 55, 34 and 15% of the seeds, respectively, at this depth.

**Figure 5.4.** Effect of tillage systems (minimum tillage, MINT and no-till, NT) on vertical seed distribution at the Roseworthy Campus in 2003 and 2005. Vertical bars with caps represent standard error.



Figure 5.5. Effect of tillage systems (DayBreak, K-Hart, narrow point and Ribbon seeder) on vertical seed distribution at Minlaton in 2003 and 2005. Vertical bars with caps represent standard error. Yenish et al. (1996) reported that in the NT system more than 90% of glass beads, representing seeds, remained in the top 2 cm of the soil profile, but that mouldboard ploughing buried 50 to 60% of the seeds to a depth of 11 to 16 cm. From our studies, it appears that under low soil disturbance systems, a large proportion of the weed seed bank will be left on the soil surface after sowing.

#### 5.4.2 Seedling Emergence Under Different Tillage Systems

At the Roseworthy Campus in 2003, the estimated initial seed bank was similar (P > 0.05) between NT (1610 seeds/m<sup>2</sup>) and MINT (1760 seeds/m<sup>2</sup>) treatments. The MINT plots had significantly greater seedling emergence than the NT plots at all seedling census dates (Figure 5.6). The maximum seedling emergence estimated from the fitted model (Equation 5.1) was threefold greater under MINT than under NT (Table 5.1; Figure 5.6). The start of seedling emergence was delayed under NT compared with MINT and the rate of seedling emergence (slope,  $E_{rate}$ ) was also greater under MINT. Conversely, not only was seedling emergence lower under NT, the time taken for 50% seedling emergence ( $t_{50}$ ) was also 7 days longer than with MINT.



**Figure 5.6.** Seedling emergence pattern of annual ryegrass as influenced by minimum tillage (MINT, solid line) and no-till (NT, dotted line) during 2003 at the Roseworthy Campus. Vertical bars represent standard error. Lines represent the functional three-parameter logistic model fitted to the data. Seedling emergence of annual ryegrass between tillage systems was significant at all dates of observation according to ANOVA (P = 0.05).

**Table 5.1.** Seedling emergence pattern of annual ryegrass as influenced by minimum tillage (MINT) and no-till (NT) at the Roseworthy Campus in 2003 and 2005. Parameter estimates are followed by standard error (SE) in parentheses. Seedling emergence data were fitted to a functional three-parameter logistic model.  $E_{max}$  is the maximum seedling emergence (plants/m<sup>2</sup>),  $t_{50}$  is the time to reach 50% of maximum seedling emergence (days) and  $E_{rate}$  indicates the slope around  $t_{50}$ .

Year	Tillage	Parameter estimates (SE)					
(Population)	-	$E_{max}$	$E_{rate}$	<i>t</i> <sub>50</sub>	$r^2$		
2003	MINT	1410.5 (73.49)	-2.9 (0.681)	14.2 (0.871)	0.997		
	NT	409.1 (0.11)	-11.7 (0.067)	21.5 (0.004)	0.999		
2005	MINT	1411.2 (60.03)	-1.3 (0.220)	7.6 (0.605)	0.999		
(RCE1)	NT	572.9 (14.22)	-2.3 (0.222)	13.4 (0.597)	0.999		
2005	MINT	1486.5 (99.43)	-1.2 (0.283)	7.9 (0.967)	0.998		
(RCE5)	NT	641.6 (11.53)	-2.3 (0.192)	11.6 (0.428)	0.999		
2005	MINT	1291.8 (18.47)	-1.6 (0.127)	7.0 (0.228)	0.999		
(RCE9)	NT	335.9 (7.15)	-2.2 (0.190)	13.1 (0.510)	0.999		
2005 (RC	MINT	1391.8 (38.37)	-1.4 (0.176)	7.2 (0.395)	0.999		
NW7)	NT	702.8 (18.84)	-2.2 (0.240)	13.0 (0.640)	0.999		

A similar pattern was observed at this site in 2005, when four different populations of annual ryegrass were included in the study. The seedling emergence response of all the populations to the two tillage systems was similar (Table 5.1; Figure 5.7). The rate of seedling emergence (slope,  $E_{rate}$ ) was greater under MINT than under NT for all populations. MINT system adequately covered the weed seed with soil and provided more favourable moisture and temperature conditions for germination and seedling establishment. In contrast, NT system left most of the seeds at the soil surface where they are prone to rapid desiccation and resulted in later seedling emergence than MINT. The maximum seedling emergence of all populations estimated from the model (Equation 5.1) was 2 to 3.8-fold greater under MINT (1290 to 1490 plants/m<sup>2</sup>) than under NT (336 to 703 plants/m<sup>2</sup>). Again the time taken for 50% seedlings to emerge ( $t_{50}$ ) was lower under MINT (7 to 8 days) than under NT (12 to 13 days).

At the Minlaton site, the seedling emergence pattern of annual ryegrass was studied under direct-drilled disc (DB and KH) and tine-based (NP and RS) seeding systems in 2003 and 2005. The seedling emergence response of annual ryegrass to the tillage systems was similar between years (Figure 5.8). In both years at the Minlaton site, plots sown with the disc systems (DB and KH) had significantly lower seedling emergence of annual ryegrass than those sown with the tine systems (Table 5.2; Figure 5.8). NP, which gives intermediate soil disturbance, had greater maximum seedling emergence (997 to 1013 plants/m<sup>2</sup>) than DB (701 to 738 plants/m<sup>2</sup>) and KH (751 to 752 plants/m<sup>2</sup>), but lower than RS (1220 to 1294 plants/m<sup>2</sup>). In both years, the rate of seedling emergence (slope,  $E_{rate}$ ) was greater under the tine than under the disc systems; however, it was statistically significant only in 2005.

**Table 5.2.** Seedling emergence pattern of annual ryegrass as influenced by Ribbon seeder (RS), narrow point (NP), K-Hart (KH) and DayBreak (DB) tillage systems at the Minlaton site in 2003 and 2005. Parameter estimates are followed by standard error (SE) in parentheses. Seedling emergence data were fitted to a functional three-parameter logistic model.  $E_{max}$  is the maximum seedling emergence (plants/m<sup>2</sup>),  $t_{50}$  is the time to reach 50% of maximum seedling emergence (days) and  $E_{rate}$  indicates the slope around  $t_{50}$ .

Year	Tillage	Parameter estimates (SE)					
		$E_{max}$	$E_{rate}$	<i>t</i> <sub>50</sub>	$r^2$		
2003	RS	1219.9 (25.23)	-2.3 (0.472)	11.1 (0.885)	0.999		
	NP	996.7 (96.71)	-1.4 (0.722)	9.8 (1.501)	0.999		
	KH	751.3 (27.67)	-2.9 (0.467)	18.7 (0.711)	0.999		
	DB	701.4 (22.10)	-2.8 (0.452)	17.2 (0.555)	0.999		
2005	RS	1294.2 (13.91)	-4.6 (0.298)	15.4 (0.218)	0.999		
	NP	1013.3 (2.83)	-7.1 (0.149)	16.3 (0.066)	0.999		
	KH	751.7 (0.06)	-14.1 (0.016)	17.6 (0.005)	0.999		
	DB	738.4 (0.04)	-12.9 (0.021)	16.3 (0.004)	0.999		



Figure 5.7. Seedling emergence pattern of four populations of annual ryegrass as influenced by minimum tillage (MINT, solid line) and no-till (NT, dotted line) during 2005 at the Roseworthy Campus. Vertical bars represent standard error. Lines represent the functional three-parameter logistic model fitted to the data. Seedling emergence (plants/m<sup>2</sup>) data was plotted against time (days after sowing). Seedling emergence of annual ryegrass between tillage systems was significant at all dates of observation according to ANOVA (*P* = 0.05).

Lower seedling establishment under the low soil disturbance systems could be because of their tendency to leave most of the seeds at the soil surface (Figure 5.4 and 5.5) where they are prone to rapid desiccation (e.g. Mohler and Galford 1997). Predation by insects is also likely to be greater in seeds present on or near the soil surface (Mohler and Galford 1997). In contrast, the high soil disturbance systems, such as MINT at the Roseworthy Campus and RS at Minlaton, adequately covered

the weed seed with soil and provided more favourable moisture and temperature conditions for germination and seedling establishment. In contrast to our study, seedling emergence of other grassy weeds has been reported to be greater under the NT systems compared to the conventional systems (Buhler and Daniel 1988).



Figure 5.8. Seedling emergence pattern of annual ryegrass as influenced by Ribbon seeder (RS, solid line), narrow point (NP, long dash line), K-Hart (KH, medium dash line) and DayBreak (DB, dotted line) 2003 2005 and in at Minlaton. Vertical bars with caps represent standard error without and bars caps represent LSD (P = 0.05) according to ANOVA. Lines represent the functional three-parameter logistic model fitted to the data.

At the Roseworthy Campus site, plants of the RCE5 population were sampled for dry matter production at 112 DAS (Table 5.3). Dry matter of individual plants was greater in MINT than in NT. Mean dry matter was 1.02 g/plant under MINT compared to 0.75 g/plant under NT. At Minlaton, dry matter of annual ryegrass plants was measured at 75 DAS (Table 5.3). At this site, plants from NP plots had greater mean dry matter (0.25 g/plant) than those from DB plots (0.20 g/plant) and KH plots (0.18 g/plant), but less than those from RS plots (0.29 g/plant).

At both sites, the tillage systems that caused the greatest soil disturbance resulted in more vigorous annual ryegrass plants. It is likely that this difference in vigour of annual ryegrass is related to the periodicity of emergence observed in different tillage systems (Figure 5.7 and 5.8). Later emerging plants in the low soil disturbance systems (NT and discs) are likely to experience greater competition from the crop for light, water and nutrients than early cohorts. Forcella (1984) also found that earlier established plants of silvergrass (*Vulpia* spp.) infesting a wheat crop had greater dry matter than later established plants. The slower seedling emergence of annual ryegrass under reduced tillage systems might have some implications for its management in the field. It could mean that late emerging plants may be less competitive with the crop and not have a substantial impact on crop yield loss and weed seed production.

**Table 5.3.** Mean dry matter of annual ryegrass as influenced by different tillage systems at the Roseworthy Campus (112 days after crop sowing) and Minlaton (75 days after crop sowing) sites in 2005.

Roseworth	y Campus	Minlaton			
Tillage systems	Dry matter	Tillage systems	Dry matter		
	g/plant		g/plant		
Minimum tillage	1.02	Ribbon seeder	0.29		
No-till	0.75	Narrow point	0.25		
LSD 0.05	0.23	K-Hart	0.18		
		DayBreak	0.20		
		LSD 0.05	0.04		

#### 5.4.3 Seed Bank Persistence Under Different Tillage Systems

Although there were significant differences between the tillage systems in the seedling emergence of annual ryegrass at both sites (Figure 5.6, 5.7 and 5.8), these differences were not reflected in the size of the residual (viable) seed bank present at the end of the season prior to seed rain (Figure 5.9, 5.10 and 5.11). At the Roseworthy Campus site, the trend in both years was similar in terms of seed fate (Figure 5.9 and 5.10). The carryover of residual seeds (old seeds) from one year to the next was not different between tillage systems, averaging 9 to 13% (Figure 5.9), most probably because of considerably greater decay of weed seeds under NT (58%) than MINT (12%).

Similarly in 2005, seed decay in all populations was greater under NT (48 to 60%) than MINT (22 to 39%). As was the case in 2003, persistence of annual ryegrass seeds of all four populations in 2005 were similar between MINT and NT, averaging 7 to 14% of the initial seed bank. There was a small component of the seed bank that could not be recovered, possibly because of complete decay of the seeds in the field or variability of distribution in the sampled area. Given the small size of this component, it is unlikely to influence the interpretation of the results on the fate of annual ryegrass seed bank.



**Figure 5.9.** Fate of annual ryegrass seed as influenced by minimum tillage (MINT) and no-till (NT) system at the Roseworthy Campus in 2003. Asterisks represent significant differences between tillage systems by ANOVA.

In 2003 at the Minlaton site, the estimated initial seed bank of annual ryegrass was 5300, 4100, 4700 and 4700 seeds/m<sup>2</sup> under the DB, KH, NP and RS systems, respectively. The total seedling emergence from the seed bank was greater under the highest soil disturbance system, RS (26%) than under the lowest soil disturbance system, DB (13%) (Figure 5.11). Although the total seedling emergence was considerably greater at the Minlaton site in 2005, the ranking of the tillage systems was identical to 2003. The greatest seedling emergence occurred under RS (65%) followed by NP (51%) and then the disc systems (37 to 38%). Lower overall seedling establishment in 2003 as compared to 2005 could be because of presence of moderate water repellence (non-wetting) in the soil at the 2003 Minlaton site. Although the tillage systems did not affect seed bank persistence of annual ryegrass, the overall level of persistence was much greater in 2003 (24 to 30%) than in 2005 (16 to 18%). Again the water repellence in the soil at the 2003 Minlaton site is likely to have influenced the level of seed persistence.



**Figure 5.10.** Fate of seed of four populations of annual ryegrass as influenced by minimum tillage (MINT) and no-till (NT) system at the Roseworthy Campus in 2005. Asterisks represent significant differences between tillage systems by ANOVA.



**Figure 5.11.** Fate of annual ryegrass seed as influenced by Ribbon seeder (RS), narrow point (NP), K-Hart (KH) and DayBreak (DB) at Minlaton during 2003 and 2005. Bars represent LSD between tillage systems by ANOVA.

Seed persistence levels in our study are consistent with those reported by Peltzer and Matson (2002), who found that 20 to 30% of the annual ryegrass seed bank can persist into the next year. However in an earlier study, McGowan (1970) reported seed persistence levels of annual ryegrass below 1% under an undisturbed pasture. In 2003, seed decay was greater under DB (50%) than under RS (38%); however, the difference was not statistically significant (Figure 5.11). Similarly in 2005, seed decay was greater under the low soil disturbance discs (35%) followed by the intermediate soil disturbance NP (22%), and the high soil disturbance RS (13%). Greater levels of seed decay under the low soil disturbance systems could be because of an unfavourable microenvironment for seedling emergence on the soil surface or because of greater predation activity of insects on or near the soil surface (Mohler and Galford 1997). Although over the two years, the study involved natural seed bank in 2003 and an artificial seed bank in 2005, the emergence response of annual ryegrass to tillage over these two years was very similar.

# **5.5 Conclusions**

Low soil disturbance systems were found to concentrate weed seeds on or near the soil surface and resulted in lower and slower seedling emergence of annual ryegrass. In addition, the size of the plants produced was also smaller. This effect of tillage system on the vigour of annual ryegrass could influence its competitive interactions with the crop. Even though high and low soil disturbance tillage systems had large effects on seedling emergence, they did not influence the persistence of the annual ryegrass seed bank. This was due to the compensatory effect of greater seed decay under the low soil disturbance NT systems.

The information from this study will be useful for developing and validating seedling emergence and seed bank prediction models for annual ryegrass. The study also indicates that the adoption of NT is unlikely to aggravate the problem of annual ryegrass, thus giving confidence to extension agencies promoting such systems. The study also indicates soil characteristics, such as non-wetting, can affect persistence of the weed seed bank. Further research is needed to determine persistence and seeddecay levels in soils with greater levels of non-wetting. Because of the effects of tillage system on the seedling emergence, persistence and decay of seed bank could be weed species specific, research on these aspects is also needed for other important Australian cropping weeds.

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# **Chapter 6. Influence of tillage systems on efficacy of three dinitroaniline herbicides and bioavailability of trifluralin**<sup>\*</sup>

# **6.1 Abstract**

Experiments were conducted to evaluate the effect of different tillage systems and dinitroaniline herbicides on control of annual ryegrass in wheat and effect of tillage systems on loss of bioavailable trifluralin. Experiments were conducted in South Australia at the Roseworthy Campus and Minlaton on the Yorke Peninsula in 2004 and 2005. The tillage systems at the Roseworthy Campus were minimum tillage and no-till using narrow points; and the herbicides were trifluralin at 0.77 kg ai/ha and pendimethalin at 0.59 kg ai/ha. The tillage systems at Minlaton were low soil disturbance discs (DayBreak and K-Hart) and high soil disturbance tines (narrow point and Ribbon seeder); and the herbicides were oryzalin, pendimethalin and trifluralin at rate of 0.72 kg ai/ha. At both sites, the total seedling emergence of annual ryegrass was greater in the nontreated plots planted with the high soil disturbance systems compared with the low soil disturbance systems. In general, oryzalin was the least effective herbicide in controlling emergence of annual ryegrass. All herbicides were more effective in reducing the number of plants, spikes, dry matter and seed production of annual ryegrass in combination with tines than with discs. However in the absence of herbicides, plant and spike numbers, plant dry weight and seed production of annual ryegrass were significantly lower where discs were used to sow rather than tines. In the absence of herbicide, annual ryegrass was less competitive with wheat under the disc-sown systems. The response of grain yield to herbicides was greater under the tine-sown systems than the disc-sown

<sup>\*</sup> Some contents of this chapter have been accepted for publication in 'Weed Technology' and 'Weed Science' journals.

Chauhan, B.S., Gill, G. and Preston, C. Effect of tillage systems and dinitroaniline herbicides on emergence and control of rigid ryegrass (*Lolium rigidum*) in wheat (*Triticum aestivum*). Weed Technology (in press).

Chauhan, B.S., Gill, G. and Preston, C. 2006. Tillage systems affect trifluralin bioavailability in soil. Weed Science 54: 941-947.

systems. A bioassay, based on response of oat roots, was used to quantify the concentrations of bioavailable trifluralin under different tillage systems. In both years at the Roseworthy Campus, loss of bioavailable trifluralin was greater under no-till than minimum tillage; however, the rate of loss under both systems was much faster in 2004 than 2005. In 2004, bioavailable trifluralin at 12 days after sowing under minimum tillage and no-till was 55 and 33% of the initial concentration, respectively. While in 2005, bioavailable trifluralin remaining at 23 days after sowing was 86% under minimum tillage and 54% under no-till. At Minlaton in both years, bioavailable trifluralin was greater under tillage systems that provided the greatest level of soil disturbance than the lower soil disturbance systems.

# **6.2 Introduction**

Annual ryegrass (*Lolium rigidum* Gaud.) is the worst example of herbicide resistance in weeds in both Australia and the world. It has now evolved resistance to herbicides with nine different modes of action (Heap 2004). In addition, annual ryegrass has been shown to cause large reductions in the grain yield of wheat (*Triticum aestivum* L.) (Poole and Gill 1987; Smith and Levick 1974). Dinitroaniline herbicides were widely adopted in Australia for weed control but fell out of favour following the introduction of post-emergent selective herbicides. However, intensive and continuous use of post-emergent herbicides over a long period of time has resulted in selection for widespread resistance in weed populations (Llewellyn and Powles 2001). Consequently the dinitroaniline herbicides, particularly trifluralin, are now being widely used again to control annual ryegrass.

Concurrently, grain growers in Australia have rapidly adopted no-till (NT) cropping and stubble retention systems (D'Emden and Llewellyn 2004) to capture their sustainability benefits. These benefits include reduction of soil erosion, fuel and labour savings, improvement in soil water retention, reduction of evaporation, improvement in soil tilth and timeliness of crop sowing (Doran and Linn 1994; Frye 1984; Griffith et al. 1986; Triplett and VanDoren 1977). The significant increase in NT crop establishment that has occurred in recent years in Australia has changed the soil microenvironment within which the dinitroaniline herbicides must perform. Trifluralin is tightly bound to dry soil (Grover et al. 1997; Weber 1990), but is volatile when applied to a moist soil surface (Bardsley et al. 1968; Parochetti et al. 1975; Parochetti and Hein 1973) because it has a very high vapour pressure (1.99 x  $10^{-4}$  mm Hg at 29.5 C). The persistence of trifluralin under field conditions is dependent upon several factors including soil moisture, temperature, soil type, and length of time before incorporation takes place (e.g. Horowitz et al. 1974; Kennedy and Talbert 1977; Messersmith et al. 1971; Probst et al. 1967; Savage 1978).

Trifluralin is thought to dissipate primarily by photodecomposition and volatilisation (Grover et al. 1997). Trifluralin also becomes tightly bound to organic matter (Kenaga 1980) and crop residues on the soil surface under reduced tillage systems can intercept a significant part of the herbicide applied. Herbicide losses may be influenced by the amount of ground covered by crop residue, the herbicide chemical properties and the herbicide formulation (Johnson et al. 1989).

Incorporation of trifluralin in soil can reduce volatilisation and photodegradation losses (Basham and Lavy 1987; Savage and Barrentine 1969; Walker and Bond 1977). Conversely, the rate of loss can be rapid if the herbicide is not adequately incorporated into soil. For example, only 2% of the initial trifluralin amount remained after 12 weeks when the herbicide was not incorporated (Savage and Barrentine 1969). Even with incorporation, trifluralin is eventually lost from soil. In a survey conducted in the United States at the end of the growing season, most soil samples contained only 1 to 8% of the original amount of trifluralin applied (Parka and Tepe 1969).

As a result of the herbicide losses, there may be insufficient herbicide remaining to control the late emerging cohorts of annual ryegrass under NT systems. Grain growers have responded to the problems of reduced soil incorporation and increased crop residue on the surface under NT by increasing the trifluralin rate applied. Other factors could also be reducing the performance of trifluralin including evolution of resistance to this herbicide (McAlister et al. 1995); however, as grain farmers move towards greater reductions in soil disturbance at sowing, the rapid loss of trifluralin from soil is likely to become increasingly important. In addition, different NT systems are likely to provide different patterns of soil disturbance and this could

influence herbicide incorporation and hence efficacy. At present, the information on soil disturbance effects on herbicide incorporation and efficacy is limited under Australian growing conditions.

Given that NT area and herbicide resistance are likely to increase (D'Emden and Llewellyn 2004), research is needed to study the efficacy of different dinitroaniline herbicides under rapidly changing tillage systems. Therefore, the objectives of this study were to determine the effect of tillage systems and dinitroaniline herbicides on the seedling emergence and control of annual ryegrass, growth and grain yield of wheat and trifluralin bioavailability.

# **6.3 Materials and Methods**

# **6.3.1 Experiment Description**

Experiments were conducted during the growing seasons of 2004 and 2005 at two sites: on the Roseworthy Campus Farm of the University of Adelaide and near Minlaton on the Yorke Peninsula of South Australia. The climate of the experimental sites is Mediterranean, which is characterised by cool wet winters and hot dry summers. Soil characteristics in the experimental fields and the date of crop sowing are presented in Table 6.1. The rainfall data for the experimental sites in 2004 and 2005 are presented in Table 6.2.

Site	Year	pН	Texture	OC	DCS	Time of soil sampling
				-%-		——DAS——
Roseworthy	2004	7.8	Heavy clay	2.0	06 June	0, 1, 3, 5 and 12
Campus	2005	7.5	Clay loam	1.5	17 June	0, 3, 7, 11, 15 and 23
Minlaton	2004	7.9	Heavy clay	2.9	01 June	30
	2005	7.9	Heavy clay	2.9	11 July	0, 4, 8, 14 and 25

**Table 6.1.** Soil characteristics in the experimental fields, and the time of crop sowing and soil sampling for 2004 and 2005 at the Roseworthy Campus and Minlaton<sup>a</sup>.

<sup>a</sup>Abbreviations: OC, organic carbon; DCS, date of crop sowing; DAS, days after sowing.

Month	Roseworthy Campus				Minlaton		
	2004	2005	Mean <sup>a</sup>	2004	2005	Mean <sup>a</sup>	
	mm						
January	11.6	21.6	21.4	11.0	4.2	14.1	
February	7.4	14.0	19.3	3.8	8.4	16.7	
March	16.0	9.4	20.3	16.8	5.4	16.1	
April	5.0	14.6	37.0	20.8	10.0	33.1	
May	29.1	2.8	46.8	34.4	4.6	54.7	
June	94.1	101.2	52.5	88.2	50.1	64.9	
July	49.0	38.2	49.9	55.7	37.3	65.9	
August	66.8	60.8	52.8	84.4	58.9	59.9	
September	11.7	53.6	46.8	42.8	75.7	46.1	
October	5.6	114.8	43.0	6.0	75.2	36.4	
November	50.2	53.6	27.7	19.6	77.6	22.3	
December	38.6	42.1	22.9	22.6	26.8	17.3	
Total	385.1	526.7	440.4	406.1	464.2	447.5	

**Table 6.2.** Monthly rainfall (mm) at the Roseworthy Campus and Minlaton in 2004 and 2005.

<sup>a</sup>Represents mean rainfall of 130 years.

The tillage systems used at the Roseworthy Campus were minimum tillage (MINT) and NT. The MINT plots were cultivated twice to a depth of 8 to 10 cm before crop sowing. A field cultivator with narrow shanks (16-mm) and 100-mm wide sweeps was used for cultivation. In NT plots, soil disturbance was limited to the sowing operation only. Wheat cv. Krichauff was sown with a seeder fitted with knife-point soil openers (16-mm wide) in both tillage systems in 2004. In 2005, wide tines (100-mm wide sweeps at the tip of the 16-mm wide shank) were used for sowing MINT to reflect farmer practice. The MINT plots were harrowed in 2005 after sowing to incorporate the herbicides.

The herbicides used at this site were trifluralin at 0.77 kg ai/ha and pendimethalin at 0.59 kg ai/ha. The herbicides were sprayed immediately prior to sowing of wheat in

both systems and incorporated by the sowing operation. Nontreated plots were sown without prior herbicide application. The herbicides were applied by using a 5-m-wide boom sprayer mounted on a four-wheel drive all-terrain vehicle that delivered 100 L/ha spray solution through flat fan nozzles at a spray pressure of 200 kPa.

The depth of wheat sowing was targeted at 3 cm. The distance between the crop rows was 25 cm and the crop was sown at the seed rate of 90 kg/ha. Urea (100 kg/ha) and diammonium phosphate (100 kg/ha) were drilled with the wheat seed. The final area of the experimental plots in both years was 7 m<sup>2</sup> (4.0 by 1.75 m). The experiments were randomised in a split plot design with tillage systems as the main plots and herbicides as the sub-plots. There were three replicates of each treatment.

The tillage systems at Minlaton comprised two disc systems, DayBreak (DB) and K-Hart (KH); and two tine-based systems, narrow point (NP) and Ribbon seeder (RS). The soil disturbance caused by these systems was in the increasing order of DB < KH < NP < RS (for additional details of these tillage systems, refer to Chapter 5). No tillage was conducted prior to herbicide application at this site.

The herbicides used at Minlaton were oryzalin, pendimethalin and trifluralin at rate of 0.72 kg ai/ha. Although oryzalin is not currently registered for use in wheat in Australia, it was included in this study to compare its efficacy with trifluralin at equivalent rates. Nontreated plots were sown without prior herbicide application. The herbicides were applied immediately before sowing the wheat as described above.

At Minlaton, the distance between the crop rows was 25 cm and the crop was sown at 70 kg/ha. Urea (80 kg/ha) was broadcast prior to seeding and diammonium phosphate (100 kg/ha) was drilled with the wheat seed. In both years, annual ryegrass seed was spread at the rate of 500 seeds/m<sup>2</sup> before wheat was sown, as the site was free from the background seed bank of annual ryegrass. All plots were sprayed with metsulfuron methyl at 3 g ai/ha on 13 July, 2004 and 05 September, 2005 to control broadleaf weeds. The final area of the experimental plots was 15 m<sup>2</sup> (10.0 by 1.5 m) in 2004 and 3 m<sup>2</sup> (2.0 by 1.5 m) in 2005. The experiments were randomised in a strip plot design with tillage systems as the main plots and herbicides as the sub-plots. There were three replicates of each treatment.

#### **6.3.2 Annual Ryegrass Measurements**

#### 6.3.2.1 Seedling Emergence Pattern

At the Roseworthy Campus in both years, two permanent quadrats (0.5 m by 0.5 m) were placed in each nontreated plot to determine the seedling emergence pattern of annual ryegrass under the different tillage systems (MINT and NT). Annual ryegrass seedlings emerging within these quadrats were counted at 7, 14, 21, 28, 35, 42, 49 and 56 days after crop sowing (DAS).

Similarly at Minlaton, two permanent quadrats (0.4 by 0.4 m) were placed in each nontreated plot to determine the seedling emergence pattern of annual ryegrass under different tillage systems (only in 2005). Annual ryegrass seedlings emerging within these quadrats were counted at 7, 14, 21, 30 and 45 DAS. Control plots (where weed seeds were not applied) were also established and seedling emergence measured in these plots to determine the background seed bank. However, there was no seedling emergence in the control plots indicating an absence of background seed bank of annual ryegrass.

#### 6.3.2.2 Density and Growth

The efficacy of herbicides at the Roseworthy Campus was evaluated on annual ryegrass under MINT and NT system. Annual ryegrass seedlings were counted 30 DAS in four quadrats (0.4 m by 0.4 m) placed at random in each plot. Herbicide efficacy was calculated by comparing weed density in the treated plots with the nontreated plots. Annual ryegrass spikes were counted at maturity. The dry matter of annual ryegrass plants from these quadrats was taken in both years and the number of seeds produced by these plants counted.

Similarly, the herbicide efficacy at Minlaton was evaluated at 30 DAS. Two quadrats (0.5 by 0.5 m) were placed in each plot at random to determine the density of annual ryegrass. Annual ryegrass seedlings within these quadrats were counted and the efficacy of herbicides was evaluated by comparing the density in the herbicide-treated plots with the nontreated plots. Annual ryegrass spikes were counted at maturity. Due to the early shedding of seeds in 2004, the dry matter of annual

ryegrass plants from these quadrats was taken only in 2005 and the number of seeds produced by these plants determined.

#### 6.3.3 Growth and Grain Yield of Wheat

At the Roseworthy Campus, various traits of wheat were recorded during the growing season. The seedling emergence of wheat was counted at 25 DAS by randomly placing two quadrats (0.5 m by 0.5 m) in each plot. Similarly, the heads of wheat were counted at maturity by randomly placing two quadrats (0.5 m by 0.5 m) in each plot. Grains per head were determined by randomly sampling 20 heads per plot. The grain yield of wheat was determined at maturity by harvesting (03 December, 2004 and 19 December 2005) the whole plot with a small plot harvester. The 1000-grain weight was determined using the harvest grain sample.

Various morphological traits of wheat were also recorded at Minlaton during the growing seasons. The variation in seedling emergence depth of wheat influenced by the tillage systems was determined at 30 DAS by randomly sampling 104 plants per tillage treatment in 2004 and 124 plants per tillage treatment in 2005. Depth of seedling emergence was measured from the seed coat to the soil surface. The seedling emergence of wheat was determined at 30 DAS by randomly placing two quadrats (0.5 m by 0.5 m) in each plot. Similarly, the heads of wheat were counted at maturity by randomly placing two quadrats (0.5 by 0.5 m) in each plot. Grains per head were determined at maturity by harvesting (16 December, 2004) the whole plot with a small plot harvester in 2004. In 2005, the plots were harvested (17 December, 2005) by hand and the heads were threshed with a small laboratory thresher. The 1000-grain weight was determined using the harvest grain sample.

#### 6.3.4 Trifluralin Bioavailability

Several methods have been used to measure trifluralin in soils, including high performance liquid chromatography (Cabras and Melis 1991), gas chromatography (Krause and Niemczyk 1992), antibodies (Garimella et al. 2000), and <sup>14</sup>C (Golab et al. 1979). Recovery of trifluralin from soil required for analytical techniques can
vary with trifluralin concentration, extraction method and soil type (Garimella et al. 2000). In any case, weed control by trifluralin is not based on the total amount present, but its bioavailability. Therefore, a bioassay will provide important information about the bioavailability of trifluralin (Johnstone et al. 1998).

Tillage system effects on trifluralin bioavailability were determined at both sites in 2004 and 2005. As trifluralin is known to have greater vapour pressure than pendimethalin and oryzalin, and is more susceptible to rapid loss under NT, it was selected for the study of bioavailability. At the Roseworthy Campus, both tillage systems (MINT and NT) were sampled in 2004 and 2005. At Minlaton, only the DB and RS plots were sampled in 2004, whereas all four tillage systems were sampled in 2005.

# 6.3.4.1 Soil Sampling

The upper 0- to 5-cm soil layer was sampled from all replications at different times after crop sowing (Table 6.1). Ten 4.5-cm-diameter cores were randomly taken from each plot. The seeding operations only disturbed the top few centimetres of soil, so it was considered unlikely that any trifluralin was moved below 5 cm.

Four different designs of tillage implements were used at the Minlaton site, which disturbed the crop row to a different extent. Therefore, soil samples in 2005 were taken from the intra-row (within the crop rows) and inter-row (between the crop rows) spaces to determine lateral movement of trifluralin. Samples were taken immediately after sowing from the DB and RS tillage systems, which were the lowest and greatest disturbance systems, respectively. During sampling in the field, soil samples were placed in sealed-plastic bags and stored temporarily in styrofoam boxes containing ice. The soil samples were frozen at -18 C on returning from the field until analysis.

# 6.3.4.2 Bioassay Procedure

All bioassay experiments were conducted in a glasshouse at the Roseworthy Campus. Preliminary experiments were conducted to develop a suitable bioassay technique that was simple to use. Oat roots were more sensitive to trifluralin inhibition than oat shoots and with lower variability. Therefore, inhibition of root growth was selected as the bioassay criterion to study bioavailability of trifluralin.

Frozen soil samples were thawed for 6 hours at room temperature before placing in pots. The soil sample from each plot was thoroughly mixed and 550 g samples were placed in 600 ml clear-plastic pots. The pots had holes at the bottom and aluminium foil was wrapped around the pots to eliminate light effects on roots. Concurrently, a standard curve for each site in each year was developed by using nontreated soil that had been air-dried and adding known trifluralin rates from 0 to 1.0  $\mu$ g/g soil for the Roseworthy Campus bioassays and from 0 to 0.5  $\mu$ g/g soil for the Minlaton bioassays.

Six oat seeds were seeded 0.5 cm deep in the pots and a thin layer of nontreated soil was spread on the surface to prevent photodecomposition. The pots were placed in a glasshouse and arranged in a randomised complete block design with three replicates. The glasshouse temperature was fluctuated between  $25 \pm 5$  C during the day and  $15 \pm 5$  C during the night. Oat roots were washed from the soil 21 days after sowing when seedlings in the nontreated soil had reached the 2.5- to 3-leaf stage. The four most uniform seedlings from each pot were used to measure root length. Oat roots were scanned with a flatbed scanner and total root length determined with the WinRHIZO<sup>1</sup> Program and represented as root length per plant.

#### **6.3.5 Statistical Analyses**

All data were analysed using analysis of variance (ANOVA) to evaluate differences and interactions between treatments and the means were distinguished using LSD 0.05. Association between measurements was examined using simple correlation analysis. Seedling emergence values at different times under different tillage systems were fitted to a functional three-parameter logistic model using SigmaPlot 2004 (version 9.0). The model fitted was:

$$E = E_{max} / [1 + (x/t_{50})^{Erate}]$$
[6.1]

<sup>&</sup>lt;sup>1</sup>WinRHIZO Pro (Version 5.0 a), Regent Instruments Inc., 4040, rue Blain, Quebec, Canada.

where *E* is the total seedling emergence (plants/m<sup>2</sup>) at time *x*,  $E_{max}$  is the maximum seedling emergence (plants/m<sup>2</sup>),  $t_{50}$  is the time to reach 50% of maximum seedling emergence (days) and  $E_{rate}$  indicates the slope. Parameter estimates were compared by using a two-tailed *t* test (*P* = 0.05).

An exponential decay curve of the form:

$$y = a^* e^{-bx}$$
 [6.2]

was fitted to the mean root length of plants grown at known herbicide concentrations (standards) by using SigmaPlot, where *y* represents the root length at bioavailable concentration of herbicide *x*, and *a* and *b* are the fitted constants. Bioavailable concentrations of herbicide under different tillage treatments over time were estimated by fitting the data for root length to Equation 6.2. The estimated bioavailable herbicide concentrations for each sample were converted to percent of the original amount applied. Assuming a bulk soil density of 1.4 g/cm<sup>3</sup> (Rainbow 2000), the doses of applied trifluralin concentration were calculated to be equivalent to 0.51 µg/g soil at the Roseworthy Campus and 0.48 µg/g soil at Minlaton. The data were analysed by using two-way ANOVA in a randomised block design with tillage and time (DAS) as the two factors.

Because of a lack of homogeneity of variance between years, data were not combined and are presented separately by year. No transformations were found necessary. Genstat version 6 was used for statistical analysis of data (Genstat 5 Committee 1993).

## **6.4 Results and Discussion**

### **6.4.1 Annual Ryegrass Measurements**

#### 6.4.1.1 Seedling Emergence Pattern

At the Roseworthy Campus, the seedling emergence pattern of annual ryegrass was studied in 2004 and 2005 in the nontreated plots of the MINT and NT systems. Regardless of the tillage system and year, seedlings of annual ryegrass emerged only until 35 DAS (Figure 6.1). In both years, MINT had significantly greater seedling emergence than NT at all seedling census dates. The maximum seedling emergence

estimated from the fitted model (Equation 6.1) was 2 to 4-fold greater under MINT (309 to 736 plants/m<sup>2</sup>) than under NT (153 to 190 plants/m<sup>2</sup>).

The start of seedling emergence was delayed under NT compared with MINT and the rate of seedling emergence (slope,  $E_{rate}$ ) was also greater under MINT. Conversely, not only was seedling emergence lower under NT, the time taken for 50% seedling emergence ( $t_{50}$ ) was also 4 to 6 days longer than with MINT. The difference in seedling emergence between MINT and NT was greater in 2005 than in 2004, which could be a result of different soil texture or climatic conditions after sowing. In 2005, the soil was lighter textured near the surface (Table 6.1) which may have caused seeds on the surface under NT to dry out more rapidly than seeds buried and insulated by the soil under MINT.



**Figure 6.1.** Seedling emergence pattern of annual ryegrass as influenced by minimum tillage (MINT, solid line) and no-till (NT, dotted line) in 2004 and 2005 at the Roseworthy Campus. Vertical bars represent standard error. Lines represent the functional three-parameter logistic model fitted to the data.

At Minlaton, the seedling emergence pattern of annual ryegrass was studied only in 2005 in the nontreated plots of all the tillage systems. Regardless of the tillage system, seedlings of annual ryegrass emerged only until 30 DAS (Figure 6.2). Plots sown with the low soil disturbance disc systems (DB and KH) had lower seedling

emergence of annual ryegrass than those sown with the high soil disturbance tine systems (NP and RS). NP had greater maximum seedling emergence (202 plants/m<sup>2</sup>) than DB (148 plants/m<sup>2</sup>) and KH (160 plants/m<sup>2</sup>) but lower than RS (292 plants/m<sup>2</sup>). Not only was seedling emergence greater in the tine-sown treatments, the rate of seedling emergence ( $E_{rate}$ ) was also greater under these systems (RS, -5.3 and NP, -7.2) as compared to the disc-sown systems (KH, -15.6 and DB, -16.8).



**Figure 6.2.** Seedling emergence pattern of annual ryegrass as influenced by the tillage systems in 2005 at Minlaton. Vertical bars represent standard error. A three-parameter logistic model was fitted to the data. The regression lines are: DayBreak, E =  $148.3/[1 + (x/16.1)^{-16.8}]$ , r<sup>2</sup>=0.99; K-Hart, E =  $160.0/[1 + (x/16.9)^{-15.6}]$ , r<sup>2</sup>=0.99; narrow point, E =  $202.3/[1 + (x/15.1)^{-7.2}]$ , r<sup>2</sup>=0.99 and Ribbon seeder, E =  $291.9/[1 + (x/14.3)^{-5.3}]$ , r<sup>2</sup>=0.99.

Seedling emergence behaviour of annual ryegrass in response to different tillage systems was consistent with studies reported in Chapter 5. Lower seedling establishment under the low soil disturbance systems (NT or discs) could be due to their tendency to leave most of the seeds on the soil surface where they are prone to rapid desiccation (e.g. Mohler and Galford 1997). Predation by insects is also likely to be greater in the seeds present on or near the soil surface (e.g. Mohler and Galford 1997). In contrast, the higher soil disturbance systems, MINT and RS, adequately covered the weed seed with soil and provided more favourable moisture and temperature conditions for germination and seedling establishment.

# 6.4.1.2 Density and Growth

At the Roseworthy Campus, annual ryegrass density was influenced by the interaction between tillage systems and herbicides in both years (Table 6.3). This response was partly due to stimulation of annual ryegrass establishment in the absence of herbicide treatment under MINT. In both years, annual ryegrass seedling emergence in the nontreated plots was greater under MINT (318 to 750 plants/m<sup>2</sup>) in comparison to NT (160 to 190 plants/m<sup>2</sup>).

**Table 6.3.** Effect of tillage systems and herbicides on annual ryegrass density in 2004 and 2005 at the Roseworthy Campus. Values in parentheses represent percent mortality of annual ryegrass plants in the herbicide-treated plots relative to the nontreated plots.

Tillage system	Herbicide			LSD <sup>a</sup> 0.05
_	Nontreated	Pendimethalin	Trifluralin	
		plants/m <sup>2</sup>		
		2004		
MINT	318	78 (75)	65 (80)	42
NT	160	80 (50)	69 (57)	42
LSD <sup>b</sup> 0.05		73		
		2005		
MINT	750	293 (61)	230 (69)	121
NT	190	107 (44)	93 (51)	131
LSD <sup>b</sup> 0.05		111		

<sup>a</sup>LSD for comparing means within a tillage system.

<sup>b</sup>LSD for comparing means within a herbicide.

Regardless of the tillage system, the herbicides provided 44% or greater control of annual ryegrass compared to the nontreated treatment. In both years, trifluralin and pendimethalin provided better control of annual ryegrass under MINT than NT. In 2004, herbicides provided 75 to 80% and 50 to 57% control of annual ryegrass under MINT and NT, respectively; and in 2005, control under MINT and NT was 61 to 69% and 44 to 51%, respectively.

At Minlaton, annual ryegrass density was significantly influenced by the interaction between tillage systems and herbicides in both years (Table 6.4); however, this was predominantly due to the large differences in the number of annual ryegrass seedlings between the different tillage systems in the nontreated plots. In both years in the nontreated plots, tillage systems had a significant effect on annual ryegrass seedling emergence. In 2004 and 2005, RS had the greatest density of annual ryegrass (223 to 280 plants/m<sup>2</sup>) followed by NP (152 to 196 plants/m<sup>2</sup>) and discs (127 to 168 plants/m<sup>2</sup>); however, in 2005, the density of annual ryegrass was similar between KH and NP. In these plots, annual ryegrass densities were greater in 2005 than in 2004.

**Table 6.4.** Effect of tillage systems and herbicides on annual ryegrass density in 2004 and 2005 at Minlaton. Values in parentheses represent percent mortality of annual ryegrass plants in the herbicide-treated plots relative to the nontreated plots.

Tillage system	Herbicide				LSD <sup>a</sup>
	Nontreated	Oryzalin	Pendimethalin	Trifluralin	0.05
		pla	ants/m <sup>2</sup>		
			2004		
DayBreak	127	93 (27)	30 (76)	22 (83)	
K-Hart	132	78 (41)	33 (75)	25 (81)	10
Narrow point	152	68 (55)	12 (92)	13 (91)	12
Ribbon seeder	223	68 (70)	23 (90)	8 (96)	
LSD <sup>b</sup> 0.05			-11		
			2005		
DayBreak	154	47 (69)	37 (76)	31 (80)	
K-Hart	168	48 (71)	34 (80)	30 (82)	24
Narrow point	196	39 (80)	22 (89)	20 (90)	54
Ribbon seeder	280	56 (80)	23 (92)	14 (95)	
LSD <sup>b</sup> 0.05			-35		

<sup>a</sup>LSD for comparing means within a tillage system.

<sup>b</sup>LSD for comparing means within a herbicide.

Despite the greater annual ryegrass density, overall control from the herbicides was similar or greater in 2005 than in 2004 (Table 6.4). In general, trifluralin and pendimethalin provided 75% or greater control of annual ryegrass compared to the nontreated treatment. Oryzalin was particularly ineffective when used with discs in 2004 (26 to 41% control). Although oryzalin performance was somewhat better in 2005, it still tended to be less effective than the other two herbicides. In both years, the control of annual ryegrass by all the herbicides was greater in the high soil disturbance tine-sown treatments (55 to 96%) than in the low soil disturbance disc-sown treatments (26 to 83%).

Trifluralin has a high vapour pressure and is thought to dissipate primarily by photodecomposition and volatilisation (Grover et al. 1997). Pendimethalin and oryzalin have lower volatility than trifluralin and may persist in the soil for a longer time (Harvey 1974). Under low soil disturbance systems such as discs, most of the applied herbicide might have remained on the soil surface even after the sowing operation, where it might be susceptible to loss through volatilisation and photodecomposition. Herbicide loss may have been lower under the high soil disturbance tillage systems due to greater soil coverage of the herbicides in the interrow space, which in turn might have reduced the losses due to volatilisation and photodecomposition. In a previous study, incorporation of trifluralin was shown to increase its persistence in the soil (Savage and Barrentine 1969). Similarly, Walker and Bond (1977) found pendimethalin to be more persistent when incorporated (80% remaining after 20 weeks) than when applied to the soil surface (20% remaining). Thus, tillage systems that incorporate these herbicides into the soil may increase the duration of efficacy on weeds.

In both years, spike density, dry matter and seed production of annual ryegrass at the Roseworthy Campus were significantly influenced by the interaction between tillage systems and herbicides (Table 6.5, 6.6 and 6.7). In 2004, these interaction effects were mainly due to the large difference in these parameters between the nontreated plots of MINT and NT. In 2005, the interaction effects were predominantly due to the large difference in these parameters between the pendimethalin and trifluralin-treated treatments.

**Table 6.5.** Effect of tillage systems and herbicides on annual ryegrass spikes in 2004 and 2005 at the Roseworthy Campus. Values in parentheses represent percent decrease of annual ryegrass spikes in the herbicide-treated plots relative to the nontreated plots.

Tillage system	Herbicide			LSD <sup>a</sup> 0.05
_	Nontreated	Pendimethalin	Trifluralin	_
		spikes/m <sup>2</sup>		
		2004		
Minimum tillage	478	83 (83)	74 (85)	20
No-till	274	93 (66)	75 (73)	20
$LSD^{b} 0.05$		24		
		2005		
Minimum tillage	890	311 (65)	245 (72)	07
No-till	251	120 (52)	108 (57)	07
LSD <sup>b</sup> 0.05		76		

<sup>a</sup>LSD for comparing means within a tillage system.

<sup>b</sup>LSD for comparing means within a herbicide.

In the nontreated plots, the response of annual ryegrass spikes to the tillage systems (MINT > NT) was very similar between the years (Table 6.5). In general, trifluralin (57 to 85%) provided similar or greater control of annual ryegrass spikes than pendimethalin (52 to 83%). As was the case with weed density, the reduction in annual ryegrass spikes/m<sup>2</sup> by the herbicides was greater under the high soil disturbance MINT system (65 to 85%) than the low soil disturbance NT system (52 to 73%). This response was partly due to the greater number of annual ryegrass spikes in the nontreated plots of MINT.

Dry matter  $(g/m^2)$  of annual ryegrass plants at the Roseworthy Campus was taken at maturity in both years and was greater in the nontreated plots of both tillage systems compared to the herbicide-treated plots (Table 6.6). In general, the control was greater in 2004 compared to 2005, and was greater under MINT (33 to 70%) than under NT (26 to 58%).

**Table 6.6.** Effect of tillage systems and herbicides on annual ryegrass dry matter in 2004 and 2005 at the Roseworthy Campus. Values in parentheses represent percent decrease of annual ryegrass dry matter in the herbicide-treated plots relative to the nontreated plots.

Tillage system	Herbicide			LSD <sup>a</sup> 0.05
_	Nontreated	Pendimethalin	Trifluralin	
		-dry matter (g/m <sup>2</sup> )-		
		2004		
Minimum tillage	155.4	49.8 (68)	45.9 (70)	0 0
No-till	101.7	52.1 (49)	42.9 (58)	0.0
LSD <sup>b</sup> 0.05		7.5		
		2005		
Minimum tillage	272	182 (33)	155 (43)	22
No-till	95	70 (26)	66 (31)	25
LSD <sup>b</sup> 0.05		35		

<sup>a</sup>LSD for comparing means within a tillage system.

<sup>b</sup>LSD for comparing means within a herbicide.

The number of annual ryegrass seeds produced was greater in the nontreated plots of both tillage systems (MINT and NT) compared to the herbicides-treated plots (Table 6.7). In 2004 and 2005, more annual ryegrass seed was set in the nontreated plots of MINT (10470 to 34480 seeds/m<sup>2</sup>) compared to NT (6394 to 9997 seeds/m<sup>2</sup>). In 2004, seeds produced under trifluralin and pendimethalin treatments were similar between the tillage systems whereas in 2005, a greater number of seeds were produced under MINT compared to NT in these treatments. Overall, the seed production of annual ryegrass was 36 to 81% lower in the herbicide-treated plots compared to the nontreated plots.

**Table 6.7.** Effect of tillage systems and herbicides on annual ryegrass seed production in 2004 and 2005 at the Roseworthy Campus. Values in parentheses represent percent decrease of annual ryegrass seed production in the herbicide-treated plots relative to the nontreated plots.

Tillage system	Herbicide			LSD <sup>a</sup> 0.05
-	Nontreated	Pendimethalin	Trifluralin	_
		-seed production/m	l <sup>2</sup>	
		2004		
Minimum tillage	10470	2297 (78)	1968 (81)	720
No-till	6394	2557 (60)	2179 (66)	129
LSD <sup>b</sup> 0.05		600		
		2005		
Minimum tillage	34480	18932 (45)	15283 (56)	1017
No-till	9997	6350 (36)	6227 (38)	1917
LSD <sup>b</sup> 0.05		2426		

<sup>a</sup>LSD for comparing means within a tillage system.

<sup>b</sup>LSD for comparing means within a herbicide.

At Minlaton, spike density, dry matter and seed production of annual ryegrass was significantly influenced by the interaction between tillage systems and herbicides (Table 6.8 and 6.9). These interaction effects were mainly due to the large difference between these parameters in the nontreated and herbicide-treated plots sown with the tines as compared to the discs.

In the nontreated plots, the effect of the different tillage systems (RS > NP > KH = DB) on the number of annual ryegrass spikes was very similar between the years (Table 6.8). Where sowing discs were used, on average annual ryegrass produced 0.7 to 1.2 spikes/plant. Where times were used for sowing, the number of annual ryegrass spikes produced by each plant was greater (1.8 to 2.9 spikes/plant). The difference could have been due to reduced competitive ability of annual ryegrass caused by the delay in seedling emergence under the disc-sown systems. Overall, oryzalin provided a less consistent reduction (20 to 88%) in annual ryegrass spikes than pendimethalin

and trifluralin (67 to 96%). As was the case with weed density, the reduction in annual ryegrass spikes/m<sup>2</sup> was greater under times than the disc systems (Table 6.8).

Tillage system		He	rbicide		LSD <sup>a</sup>
	Nontreated	Oryzalin	Pendimethalin	Trifluralin	0.05
		spi	ikes/m²		
			2004		
DayBreak	135	108 (20)	43 (68)	33 (75)	
K-Hart	152	95 (37)	47 (69)	32 (79)	34
Narrow point	307	83 (73)	22 (93)	25 (92)	54
Ribbon seeder	403	87 (79)	48 (88)	17 (96)	
LSD <sup>b</sup> ( <i>P</i> =0.05)			-32		
			2005		
DayBreak	115	41 (64)	37 (67)	31 (73)	
K-Hart	121	59 (52)	36 (70)	32 (74)	10
Narrow point	565	68 (88)	43 (92)	35 (94)	19
Ribbon seeder	620	88 (86)	56 (91)	39 (94)	
LSD <sup>b</sup> ( <i>P</i> =0.05)			-18		

**Table 6.8.** Effect of tillage systems and herbicides on annual ryegrass spike number in 2004 and 2005 at Minlaton. Values in parentheses represent percent decrease of annual ryegrass spikes in the herbicide-treated plots relative to the nontreated plots.

<sup>a</sup>LSD for comparing means within a tillage system.

<sup>b</sup>LSD for comparing means within a herbicide.

Dry matter of annual ryegrass plants was taken at maturity in 2005 and was greater in the nontreated plots of all the tillage systems compared to where dinitroaniline herbicides were used (Table 6.9). Overall, the control of annual ryegrass dry matter by dinitroaniline herbicides was greater than 70%. However, the control was greater with the tine-sown systems (88 to 94%) than in the disc-sown systems (73 to 85%).

Late emerging plants in the low soil disturbance systems may have experienced greater competition with the crop for light, water and nutrients. Consequently, those

plants may have produced less productive spikes and dry matter. Forcella (1984) found that earlier established plants of silvergrass (*Vulpia spp.*) infesting a wheat crop had greater dry matter than the later established plants.

**Table 6.9.** Effect of tillage systems and herbicides on annual ryegrass dry matter and seed production at maturity in 2005. Values in parentheses represent percent decrease in dry matter and seed production of annual ryegrass in the herbicide-treated plots relative to the nontreated plots.

Tillage system		Herbicide			
	Nontreated	Oryzalin	Pendimethalin	Trifluralin	0.05
	dry matter (g/m <sup>2</sup> )				
DayBreak	50.4	13.7 (73)	12.0 (76)	7.8 (85)	
K-Hart	49.8	9.3 (81)	13.5 (73)	8.0 (84)	0.1
Narrow point	180.4	22.5 (88)	12.3 (93)	13.0 (93)	0.1
Ribbon seeder	216.6	24.4 (89)	12.9 (94)	16.6 (92)	
LSD <sup>b</sup> 0.05			-6.6		
		seed pr	oduction/m <sup>2</sup>		
DayBreak	6720	3323 (51)	1762 (74)	1308 (86)	
K-Hart	5273	1597 (70)	2091 (60)	1350 (74)	10499
Narrow point	30013	5720 (81)	3020 (90)	1912 (94)	10400
Ribbon seeder	28533	1521 (95)	1349 (95)	1305 (95)	
LSD <sup>b</sup> 0.05		9	9809		

<sup>a</sup>LSD for comparing means within a tillage system.

<sup>b</sup>LSD for comparing means within a herbicide.

The number of annual ryegrass seeds produced under different herbicide treatments was similar in all the tillage systems (Table 6.9). However, more annual ryegrass seed was set in the nontreated plots where tines were used for sowing (28500 to 30000 seeds/m<sup>2</sup>) compared to discs (5300 to 6700 seeds/m<sup>2</sup>). Overall, the seed production of annual ryegrass was 51 to 95% lower in the herbicide-treated plots as compared to the nontreated plots. Due to low dry matter of annual ryegrass in the disc-sown treatments, the seed set by annual ryegrass was also much lower in these

systems. The low seed set under these systems could be exploited effectively by using other more suitable herbicides, which can manage this small, but significant, amount of seed set.

# 6.4.2 Growth and Grain Yield of Wheat

The effect of tillage systems and herbicides on various yield-attributing characters of wheat was measured at the Roseworthy Campus in both years. None of the yield attributing character was influenced by the interaction between tillage and herbicide.

The seedling emergence of wheat was not influenced by the tillage systems in either year of the experiment (Table 6.10 and 6.11). Although a reduction of seedling emergence was observed in the herbicide-treated plots in both years, the reduction was significant only in 2004. The observed herbicide phytotoxicity on wheat seedling emergence could be due to rainfall received immediately after planting, which might have caused herbicide movement into the furrows and increased exposure of wheat to the herbicides.

Main factor	Seedling	Grains/head	1000-grain	Heads	Grain yield
	emergence		weight		
	-plants/m <sup>2</sup> -	—no—	g	no/m²	-kg/ha-
Tillage					
MINT	202	46.6	32.91	348	3032
NT	224	44.6	32.55	363	3239
LSD 0.05	NS	NS	NS	NS	153
Herbicide					
Nontreated	274	45.2	33.35	313	2882
Pendimethalin	191	46.0	32.60	370	3243
Trifluralin	174	45.7	32.25	383	3282
LSD 0.05	28	NS	NS	40	196

**Table 6.10.** Effect of tillage systems and herbicides on wheat traits in 2004 at the

 Roseworthy Campus.

Main factor	Seedling	Grains/head	1000-grain	Heads	Grain yield
	emergence		weight		
	-plants/m <sup>2</sup> -	—no—	g	no/m²	-kg/ha-
Tillage					
MINT	237	36.0	30.17	262	2364
NT	261	34.6	30.15	322	2763
LSD 0.05	NS	NS	NS	NS	106
Herbicide					
Nontreated	274	35.2	30.15	264	2321
Pendimethalin	240	36.0	30.54	291	2634
Trifluralin	232	34.7	29.79	323	2735
LSD 0.05	NS	NS	NS	41	253

**Table 6.11.** Effect of tillage systems and herbicides on wheat traits in 2005 at the Roseworthy Campus.

The grains per head and 1000-grain weight of wheat were not influenced by either of the main factors, tillage or herbicide (Table 6.10 and 6.11). The number of crop heads was not influenced by the tillage systems in either year of the experiment (Table 6.10 and 6.11). On the other hand, an increase in the number of crop heads was observed in the herbicide-treated plots compared to the nontreated plots. The greater density of annual ryegrass plants in the nontreated treatments was likely to have provided significant competition to wheat and this might have reduced the number of crop heads in the nontreated plots.

The grain yield of wheat was influenced by the tillage systems and herbicides in both years (Table 6.10 and 6.11). In 2004 and 2005, the grain yield was greater in the NT treatment (2763 to 3239 kg/ha) compared to the MINT treatment (2364 to 3032 kg/ha). Regardless of the year, the grain yield was greater in the herbicide-treated plots (2634 to 3282 kg/ha) compared to the nontreated plots (2321 to 2882 kg/ha); however, the yield was similar between the pendimethalin and trifluralin-treated plots. As mentioned earlier, the greater density of annual ryegrass plants in the

nontreated plots compared to the herbicide-treated plots might have provided greater competition to wheat and significantly reduced yield in the nontreated plots.

Out of all the wheat traits studied in 2004 and 2005, the density of crop heads was positively and strongly correlated (P < 0.01) with the grain yield (Table 6.12). In both years, the grain yield was negatively correlated (P < 0.01) with the measured traits of annual ryegrass (plants/m<sup>2</sup>, spikes/m<sup>2</sup>, dry matter/m<sup>2</sup> and seed production/m<sup>2</sup>) thus providing evidence for competitive interactions with wheat.

Trait	Grain yield		
	2004	2005	
Wheat heads/m <sup>2</sup>	0.72*	0.83*	
Grains/head	-0.09	-0.20	
1000-grain weight (g)	-0.45	0.05	
Annual ryegrass plants/m <sup>2</sup>	-0.81*	-0.77*	
Annual ryegrass spikes/m <sup>2</sup>	-0.84*	-0.79*	
Annual ryegrass dry matter (g)/m <sup>2</sup>	-0.84*	-0.79*	
Annual ryegrass seeds/m <sup>2</sup>	-0.83*	-0.82*	

**Table 6.12.** Correlation coefficients between wheat and annual ryegrass traits and wheat grain yield at the Roseworthy Campus.

\**P* < 0.01.

At Minlaton, the effect of tillage systems on the depth of wheat seedling emergence was studied in 2004 and 2005. The mean seedling emergence depth of wheat was greater in the RS system (49 to 55 mm) than in the other tillage systems (24 to 34 mm) (Table 6.13). Although the sowing depth was targeted at 30 mm in all the tillage systems, there was greater variation of seedling emergence in the RS system compared to the other systems (Figure 6.3). The median depth of seedling emergence was also greater in the RS system. The depth of seedling emergence might influence the competitive ability of crops against weeds (Cousens and Moss 1990). Wheat seedling emergence was not influenced by the tillage or herbicide treatments in either year of the experiment (Table 6.14 and 6.15); however, there was a trend of lower wheat seedlings in RS.

Tillage system	Depth		
_	2004	2005	
	m	m	
DayBreak	27.4	28.3	
K-Hart	25.9	24.4	
Narrow point	30.2	33.5	
Ribbon seeder	54.7	48.8	
LSD 0.05	—3.1—	—3.4—	

**Table 6.13.** Mean depth (mm) of wheat seedling emergence influenced by the tillagesystems in 2004 (n=104 plants) and 2005 (n=124 plants) at Minlaton.



Figure 6.3. Influence of tillage systems on the variation of seedling emergence depth of wheat in 2004 and 2005 at Minlaton. Box plots in which the bottom and top lines are located at the 25th and 75th percentiles of the distribution, showing range from which 50% of seedling emergence occurred (box) and median depth of seedling emergence (line within the box), whiskers below and above the box indicate the 10th and 90th percentiles; and solid circle indicate the 5th and 95th percentile.

Main factor	Seedling Grains/head		1000-grain
	emergence		weight
	-plants/m <sup>2</sup> -	no	g
Tillage			
DayBreak	223	40.5	29.85
K-Hart	263	39.6	29.67
Narrow point	230	42.1	30.21
Ribbon seeder	218	42.5	30.13
LSD 0.05	NS	NS	NS
Herbicide			
Oryzalin	254	40.4	30.45
Pendimethalin	219	40.7	29.81
Trifluralin	205	41.7	29.90
Nontreated	256	42.0	29.70
LSD 0.05	NS	NS	NS

**Table 6.14.** Effect of tillage systems and herbicides on wheat traits in 2004 at  $Minlaton^{a}$ .

<sup>a</sup>Abbreviations: NS, non-significant.

The density of wheat heads was significantly influenced by the interaction between tillage systems and herbicides in both years (Table 6.16). The interaction observed in 2004 could be due to the lowest density of wheat heads in the nontreated plots of RS (183 heads/m<sup>2</sup>). In 2005, the trifluralin-treated treatments had the greatest density of wheat heads under NP (393 heads/m<sup>2</sup>), which may have contributed to the interaction observed.

Compared to the nontreated plots in both years, the use of herbicides increased the wheat head density by up to 23% in the disc systems and by up to 31% in the tine systems (Table 6.16). Overall, the response of head density to herbicides was greater in the tine-sown systems than the disc-sown systems. This response could be due to lower number of wheat heads in the nontreated plots of tines, especially RS, compared to the discs.

Puckridge and Donald (1967) in an earlier study found that the optimum density of wheat for grain yield to be between 35 and 180 plants/m<sup>2</sup>, and emphasized the compensating effect of wheat heads per unit area at these densities. This compensating effect of heads produced by wheat was observed in the present study. The number of wheat seedling was lower to some extent in 2005 than in 2004, but the number of wheat heads was greater in 2005 than in 2004 (Figure 6.16).

Main factor	Seedling	Grains/head	1000-grain
	emergence		weight
	-plants/m <sup>2</sup> -	—no—	g
Tillage			
DayBreak	150	25.0	31.21
K-Hart	163	25.5	30.53
Narrow point	158	27.0	30.06
Ribbon seeder	140	25.8	30.76
LSD 0.05	NS	NS	0.54
Herbicide			
Oryzalin	156	26.5	30.12
Pendimethalin	143	25.4	30.83
Trifluralin	142	25.7	30.67
Nontreated	171	25.8	30.94
LSD 0.05	NS	NS	NS

**Table 6.15.** Effect of tillage systems and herbicides on wheat traits in 2005 at Minlaton<sup>a</sup>.

<sup>a</sup>Abbreviations: NS, non-significant.

The size of wheat heads (grains/head) was not influenced by either tillage or herbicide (Table 6.14 and 6.15). However, the head size was smaller in 2005 (25 to 27 grains/head) than in 2004 (40 to 43 grains/head). The 1000-grain weight of wheat was not influenced by either the tillage or herbicide in 2004 (Table 6.14). While in 2005, the 1000-grain weight was influenced by the tillage systems (Table 6.15). NP

had the smallest size of grain, which was statistically similar to the grain size of wheat under KH.

Tillage system	Herbicide				LSD <sup>a</sup>
	Nontreated	Oryzalin	Pendimethalin	Trifluralin	0.05
		t	no/m²	· · · · · · · · · · · · · · · · · · ·	
			2004		
DayBreak	217	281 (23)	234 (7)	218 (0)	
K-Hart	246	312 (21)	282 (13)	282 (13)	27
Narrow point	229	285 (20)	273 (16)	278 (18)	57
Ribbon seeder	183	234 (22)	250 (27)	236 (22)	
LSD <sup>b</sup> 0.05			_47		
			<b>2</b> 00 <b>5</b>		
			2005		
DayBreak	313	370 (15)	368 (15)	359 (13)	
K-Hart	320	352 (9)	373 (14)	378 (15)	33
Narrow point	269	330 (18)	349 (23)	393 (31)	33
Ribbon seeder	267	317 (16)	336 (21)	313 (15)	
LSD <sup>b</sup> 0.05			-31		

**Table 6.16.** Effects of tillage systems and herbicides on heads of wheat in 2004 and 2005 at Minlaton. Values in parentheses represent per cent increase in the number of heads of wheat produced in the herbicide-treated plots over the nontreated plots.

<sup>a</sup>LSD for comparing means within a tillage system.

<sup>b</sup>LSD for comparing means within a herbicide.

Grain yield was significantly influenced by the interaction between tillage systems and herbicides in both years (Table 6.17). The interaction in 2004 may be due to the lowest grain yield in the nontreated plots of RS (2424 kg/ha). Across the trifluralin-treated plots, the greatest grain yield was achieved under NP (3918 kg/ha), which may have also contributed to the interaction observed. Again in 2005, the trifluralin-treated plots had the greatest grain yield under NP (3115 kg/ha).

Tillage system	Herbicide				LSD <sup>a</sup>
	Nontreated	Oryzalin	Pendimethalin	Trifluralin	0.05
		k	xg/ha		
			2004		
DayBreak	2882	3560 (24)	3102 (8)	3076 (7)	
K-Hart	3292	3912 (19)	3741 (14)	3539 (8)	100
Narrow point	3294	3917 (19)	3773 (15)	3918 (19)	132
Ribbon seeder	2424	3507 (45)	3278 (35)	3391 (40)	
LSD <sup>b</sup> 0.05			-66		
		,	2005		
DayBreak	2478	2841 (15)	2837 (15)	2829 (14)	
K-Hart	2508	2821 (13)	2860 (14)	2876 (15)	241
Narrow point	2216	2711 (22)	2834 (28)	3115 (41)	241
Ribbon seeder	2107	2530 (20)	2603 (24)	2513 (19)	
LSD <sup>b</sup> 0.05			274		

**Table 6.17.** Effect of tillage systems and herbicides on wheat grain yield in 2004 and 2005 at Minlaton. Values in parentheses represent percent increase in wheat grain yield in the herbicide-treated plots relative to the nontreated plots.

<sup>a</sup>LSD for comparing means within a tillage system.

<sup>b</sup>LSD for comparing means within a herbicide.

Despite differences in annual ryegrass control between herbicides, all herbicides increased the grain yield of wheat compared to the nontreated check. Compared to the nontreated plots, the use of herbicides increased wheat grain yield from 7 to 24% in the disc systems and 15 to 45% in the tined systems (Table 6.17). Overall, the response of grain yield to herbicides was greater under the tine-sown systems than the disc-sown systems. In both years, RS had the lowest grain yield (2107 to 2424 kg/ha) in the nontreated plots, and this result is consistent with the high density and dry matter of annual ryegrass that occurred in this tillage system. The yield loss caused by annual ryegrass in the disc-sown systems was not large, which was again consistent with the lower density of annual ryegrass plants, spikes and dry matter in these systems.

The number of crop heads per unit area is a good indicator of the potential crop yield. Therefore, out of all the wheat traits studied in 2004 and 2005, the number of heads was strongly and positively correlated (P < 0.01) with the grain yield (Table 6.18). The grain yield was negatively correlated with the measured traits of annual ryegrass (plants/m<sup>2</sup>, spikes/m<sup>2</sup>, dry matter/m<sup>2</sup> and seed production/m<sup>2</sup>). These results are consistent with the results of the experiments at the Roseworthy Campus and also supports previous findings on the competitive ability of annual ryegrass against wheat (Poole and Gill 1987; Smith and Levick 1974).

Trait	Grain	yield
	2004	2005
Wheat heads/m <sup>2</sup>	0.79*	0.90*
Grains/head	-0.06	0.09
1000-grain weight (g)	0.26	0.02
Annual ryegrass plants/m <sup>2</sup>	-0.54*	-0.75*
Annual ryegrass spikes/m <sup>2</sup>	-0.56*	-0.72*
Annual ryegrass dry matter (g)/m <sup>2</sup>	NR	-0.72*
Annual ryegrass seeds/m <sup>2</sup>	NR	-0.60*

**Table 6.18.** Correlation coefficients between wheat and annual ryegrass traits and wheat grain yield at Minlaton<sup>a</sup>.

<sup>a</sup>Abbreviations: NR, not recorded.

\**P* < 0.01.

# 6.4.3 Trifluralin Bioavailability

The results of the bioassay varied between sites and years. The difference in decay curves between different sites and years could be because of variations in soil properties such as texture and organic carbon content. This meant that a new standard curve for each year and site had to be developed along with the test samples using the same soil. However, there was consistency in the standard curves, with 50% inhibition of oat root growth occurring between 0.13 and 0.23  $\mu$ g trifluralin/g soil. This is similar to a previous study from Australia, where 50% inhibition of oat root growth was reported around 0.1  $\mu$ g trifluralin/g soil (Johnstone et al. 1998).

Oat root length and bioavailable trifluralin determined by bioassay under MINT and NT at the Roseworthy Campus are shown in Table 6.19 and percent bioavailability of trifluralin in Figure 6.4. Loss of bioavailable trifluralin was influenced by tillage systems in both years and was greater under NT than MINT (Figure 6.4).

**Table 6.19.** Oat root length and bioavailable trifluralin determined by bioassay under minimum tillage (MINT) and no-till (NT) at the Roseworthy Campus. The soil samples were taken at different times after crop sowing (DAS) in 2004 and 2005.

Time	Oat root	length	Bioavailable	e trifluralin
	MINT	NT	MINT	NT
DAS	cm/p	lant——	μg/g	soil——
		200	04	
0	33	33	0.50	0.51
1	35	34	0.48	0.49
3	38	62	0.46	0.30
5	52	66	0.35	0.27
12	64	89	0.28	0.17
LSD 0.05	10	)	0.0	)6
		200	05	
0	13.8	14.3	0.50	0.50
3	14.9	15.1	0.49	0.48
7	15.3	16.1	0.48	0.47
11	16.2	21.0	0.47	0.43
15	16.4	26.6	0.47	0.38
23	19.4	45.2	0.44	0.28
LSD 0.05	4.1	1	0.0	32

In 2004, the differences between tillage systems started to be significant when the soil was sampled just 3 DAS (Figure 6.4 c). At the last sampling time (12 DAS), bioavailable trifluralin compared to the initial concentration had dropped to 55% under MINT and 33% under NT. The concentration of bioavailable trifluralin in 2005 was very similar between MINT and NT until 7 DAS (Figure 6.4 d). After this





**Figure 6.4.** Exponential relationship between the dose of trifluralin and root length of oat in the bioassay in (a) 2004 and (b) 2005. Bioassays were used to determine the bioavailability of trifluralin (% of applied) under minimum tillage (MINT) and no-till (NT) in (c) 2004 and (d) 2005 at the Roseworthy Campus. The trifluralin concentration applied was equivalent to 0.51  $\mu$ g/g soil. Vertical bars represent standard error. Asterisk represents significant differences between tillage systems by ANOVA; LSD 0.05 = 12.3 in 2004 and 6.2 in 2005.

There was greater loss of bioavailable trifluralin under MINT in 2004 than in 2005, possibly due to better incorporation of trifluralin in 2005 as a result of harrowing after crop sowing. However, differences in loss of bioavailable trifluralin between the two years could also be related to environmental conditions because it was much drier after sowing in 2005 compared with 2004. The rainfall in the first 30 DAS was

only 32 mm in 2005 versus 86 mm in 2004, which would tend to reduce trifluralin losses through volatilisation in 2005.

The greater loss of bioavailable trifluralin under NT compared to MINT could have serious implications for annual ryegrass control, which can have a protracted pattern of germination in some seasons. The magnitude of herbicide loss between the two tillage systems could explain the lower efficacy of trifluralin under NT (51 to 57%) as compared to MINT (69 to 80%) (Table 6.3). Bioavailable trifluralin under NT might not have been sufficient to control the later-emerging annual ryegrass seedlings.

Soil samples at Minlaton in 2004 were taken only from DB (disc) and RS (wide tine) treatments. The DB system was identified as the least aggressive and the RS system was the most aggressive tillage systems for vertical distribution of weed seeds and therefore, soil disturbance. Oat root length and bioavailable trifluralin determined by bioassay under different tillage systems are shown in Table 6.20 and percent bioavailability of trifluralin in Figure 6.5.

In 2004, bioavailable trifluralin remaining 30 DAS was 42% of applied trifluralin under the DB system compared to 76% under the RS system (Figure 6.5 c). Soil samples for herbicide bioavailability analysis in 2005 were taken from plots sown with all four tillage implements. Bioavailable trifluralin was greater under the tillage systems that caused greatest levels of soil disturbance. Bioavailability of trifluralin in 2005 was very similar across all tillage systems until 4 DAS (Figure 6.5 d). At 8 and 14 DAS, the DB system had greater loss of bioavailable trifluralin than the other three tillage systems. At the last sampling period (25 DAS), bioavailable trifluralin was greatest under the RS (82%) and NP (78%) systems followed by KH (70%) and DB (55%).

There was much greater loss of trifluralin in 2004 compared to 2005 at the Minlaton site, which agreed with the results from the Roseworthy Campus. The rainfall was lower in the first 30 DAS in 2005 (40 mm) compared to 2004 (91 mm). As with the Roseworthy Campus site, reduced moisture at Minlaton is likely to reduce trifluralin volatilisation.

Table 6.20. Oat root length and bioavailable trifluralin determined by bioassay under
four tillage systems, Ribbon seeder (RS), narrow point (NP), K-Hart (KH) and
DayBreak (DB), at Minlaton. The soil samples were taken at different times after
crop sowing (DAS) in 2004 and 2005.

Time	Oat root length			Bioavailable trifluralin			alin	
-	RS	NP	KH	DB	RS	NP	KH	DB
DAS		cm/p]	lant——			μg/g	g soil—	
			20	004				
30	42.5	-	-	87.7	0.36	-	-	0.20
LSD 0.05		5.1	1			0.	05——	
			20	005				
0	14.0	13.1	13.5	13.5	0.47	0.48	0.48	0.48
4	14.6	14.7	17.0	17.3	0.46	0.46	0.44	0.43
8	16.1	16.7	17.2	21.4	0.45	0.44	0.43	0.39
14	17.7	19.2	19.0	35.4	0.43	0.41	0.41	0.30
25	21.3	23.6	29.2	42.7	0.39	0.38	0.34	0.27
LSD 0.05		3.6	5	_		0.	03	

Soil samples at the Minlaton site in 2005 were also taken immediately after sowing from the intra-row (i.e. within the row) and the inter-row space from the DB and RS systems to determine the amount of herbicide thrown out of the crop rows during the seeding operations. The concentrations of bioavailable herbicide in the intra-rows under DB and RS systems were 65% and 75% of the applied trifluralin at sowing (Table 6.21), indicating a significant lateral movement of the herbicide caused by the seeding implement. Consequently, the weed control within the intra-rows may be somewhat lower than that achieved in the inter-row area. The most aggressive soil disturbing tines, such as NP, are likely to throw even more herbicide-treated soil out from the crop rows and provide added safety to the crop. This explains why NT farmers from Australia have reported somewhat lower levels of weed control within the intra-rows than in the inter-rows.



**Figure 6.5.** Exponential relationship between the dose of trifluralin and root length of oat in the bioassay in (a) 2004 and (b) 2005. Bioassays were used to determine the bioavailability of trifluralin (% of applied) under DayBreak (DB), K-Hart (KH), narrow point (NP) and Ribbon seeder (RS) in (c) 2004 and (d) 2005 at Minlaton. The trifluralin concentration applied was equivalent to 0.48  $\mu$ g/g soil. Vertical bars represent standard error. Asterisk represents significant differences between tillage systems by ANOVA; LSD 0.05 = 10.0 in 2004 and 6.9 in 2005.

The low soil disturbance systems, in particular DB, would retain most of the trifluralin on the soil surface even after the sowing operation, so the herbicide would be susceptible to loss through volatilisation and photodecomposition. The loss of bioavailable trifluralin was lowest with the high soil disturbance tillage system (RS) due to greater soil coverage of the herbicide in the inter-rows, which would have reduced the losses due to volatilisation and photodecomposition in these systems.

Incorporation of trifluralin increased the persistence of this herbicide in a previous study (Savage and Barrentine 1969). With no incorporation, only 2% of the initial

amount of trifluralin was present after 12 weeks, while 26% and 40% of the applied trifluralin remained following incorporation to 2.5 to 5 cm and 7.5 to 10 cm, respectively. Thus, tillage systems that thoroughly mix the soil deeper and incorporate the chemicals may reduce the rate of loss of dinitroaniline herbicides caused by volatilisation and photodecomposition.

**Table 6.21.** Oat root length, bioavailable concentration and percent bioavailability (% of applied) of trifluralin present in the intra-row and inter-row at sowing under Ribbon seeder (RS) and DayBreak (DB) at Minlaton in 2005. The applied trifluralin concentration was equivalent to 0.48  $\mu$ g/g soil.

Tillage	Oat roo	t length		Bioavailability			
system			Triflu	Trifluralin		ortion	
	Intra-row	Inter-row	Intra-row	Inter-row	Intra-row	Inter-row	
	—cm/plant—		—µg/g	—μg/g soil—		—% of applied—	
RS	25.8	14.0	0.36	0.47	75	98	
DB	33.7	13.5	0.31	0.48	65	100	
LSD 0.05	——7.	.9	0.0	)5——	——10	0	

Trifluralin was lost rapidly under tillage systems that caused low soil disturbance in this study. Herbicide losses of this high magnitude could have serious implications for control of weeds that have a protracted germination pattern. In such situations, there may be insufficient trifluralin remaining to control late emerging weeds in the crop. As the amount of soil disturbance decreases, particularly with disc systems, the level of control offered by trifluralin can be expected to decrease.

Inadequate incorporation could be compensated in part by increased herbicide rates; however, in very low-disturbance systems, trifluralin is likely to have too short bioavailability to provide effective control of late emerging cohorts of weeds. In addition, wet conditions after sowing are likely to lead to greater loss of trifluralin than dry conditions, particularly in lower disturbance tillage systems. The interaction of level of disturbance and rainfall after sowing could be one reason for the variable performance of trifluralin in NT systems.

# **6.5 Conclusions**

The seedling emergence of annual ryegrass was greater in the high soil disturbance tillage systems compared to the low soil disturbance systems. The efficacy of different dinitroaniline herbicides, especially trifluralin on the density of annual ryegrass seedlings was lower in the low soil disturbance systems compared to the high soil disturbance systems. However, the lower efficacy was at least partly compensated by the lower annual ryegrass seedling emergence under the low soil disturbance tillage systems (e.g. discs) as compared to the high soil disturbance tillage systems (e.g. tines). The response of the grain yield to the herbicides was lower in the low soil disturbance discs than the high soil disturbance tines.

Crop residues present on the soil surface in NT systems could intercept a significant amount of these herbicides, thereby reducing their efficacy. Research is needed to investigate potential interaction between crop residues and herbicide dose under these tillage systems. The benefits of lower weed seedling emergence under the low soil disturbance discs can only be fully exploited if more effective herbicides, which are suitable for use in these systems, can be identified.

Therefore, the next chapter describes the potential of S-metolachlor for the control of annual ryegrass in wheat under no-till conditions.

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# Chapter 7. Influence of timing and dose of S-metolachlor on annual ryegrass control in wheat<sup>\*</sup>

# 7.1 Abstract

The efficacy of S-metolachlor on annual ryegrass was determined under a no-till seeding system in an experiment conducted in 2004 and 2005. S-metolachlor at the rate of 0.48 and 0.96 kg ai/ha was applied at 40 or 46, 20 or 23 days before crop sowing (DBS) and at sowing (AS) in 2004 and 2005 with the herbicides incorporated by sowing (IBS). In 2005, the herbicide was also applied AS as post sowing preemergence (PSPE). The control of annual ryegrass was greater than 80% when S-metolachlor was applied AS (IBS) or AS (PSPE). However, the application of herbicide AS (IBS and PSPE) resulted in phytotoxic effects on seedling emergence and grain yield of wheat. S-metolachlor applied at 0.96 kg/ha was more phytotoxic than 0.48 kg/ha. Application of S-metolachlor at 20 or 23 DBS provided 74 to 83% annual ryegrass control, whereas application at 40 or 46 DBS provided only 33 to 49% control. Reduction in wheat grain yield was not observed at these application times. This field study indicates that S-metolachlor at 0.48 kg/ha could be safely applied around 20 DBS to selectively control annual ryegrass in wheat.

# 7.2 Introduction

Annual ryegrass (*Lolium rigidum* Gaud.) is the most important weed of the cropping systems in southern Australia. In this region, herbicides are the main weed control technique used. As a result of the evolution of resistance to the post-emergence acetolactate synthase- and acetyl-CoA carboxylase-inhibiting herbicides in annual ryegrass (Llewellyn and Powles 2001), trifluralin (a soil-applied herbicide) is now being widely used for the control of this weed. However, cases of resistance have also been reported against this herbicide (e.g. McAlister et al. 1995). With several

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more cases of trifluralin resistance being discovered, there is a need to find an effective alternative herbicide with a different mode of action.

S-metolachlor is a chloroacetamide herbicide, which acts as a growth inhibitor in target weeds by preventing the synthesis of chlorophyll, proteins, fatty acids and lipids. This herbicide is widely used in many crops, such as corn (Zea mays L.), to control annual grasses and some annual broadleaf weeds (Mueller et al. 1999; Rouchaud et al. 1999). In Australia this herbicide is not recommended for annual ryegrass control in wheat (Triticum aestivum L.) due to phytotoxic effects on the crop when used at rates needed to control annual ryegrass. However, changing the application timing from at or immediately after planting to preplant could reduce the damage to wheat. For example, S-metolachlor could be applied well before crop sowing, so that it dissipates to a level that is safe for the crop as well as effective on annual ryegrass. There is a risk that very early application could result in rapid dissipation of the herbicide because of high temperature and dry conditions over a long period of time (Ahrens 1994), which may reduce the control of annual ryegrass significantly. However, rainfall events after the application may provide adequate incorporation into the soil and reduce herbicide losses and may also result in absorption of the herbicide by annual ryegrass seeds.

The effect of early application of S-metolachlor on the control of annual ryegrass in wheat has not been reported from field studies. However, one experiment was undertaken in a glasshouse to study the potential of this herbicide to control annual ryegrass (Walsh et al. 2004). The authors found that this herbicide could be used well before the normal crop sowing time to control annual ryegrass. However, ultraviolet light, which was filtered by the glasshouse could cause photodecomposition of this herbicide in the field. Therefore, the objectives of this experiment were to study the effect of application timing and dose of S-metolachlor on the emergence and growth of annual ryegrass in wheat and on the growth and yield of wheat.

# 7.3 Materials and Methods

### 7.3.1 Field Experiment

The experiment was conducted during the winter growing seasons of 2004 and 2005 at the Roseworthy Campus Farm of the University of Adelaide, South Australia. The experiment was conducted in different fields of the farm each year. The monthly rainfall data for 2004 and 2005 are shown in Chapter 6 (Table 6.2). The soil characteristics and the date of crop sowing and harvesting are shown in Table 7.1.

**Table 7.1.** Soil characteristics and dates of crop sowing and harvesting in 2004 and 2005.

Parameter	2004	2005
Soil texture	Heavy clay	Clay loam
рН	7.8	7.5
Organic matter (%)	3.4	2.6
Date of crop sowing	06 June	17 June
Date of crop harvesting	03 December	19 December

Wheat cv. Krichauff was sown under no-till conditions with 16-mm wide knife-point openers. The herbicide S-metolachlor was applied at different times at the rate of 0 (nontreated), 0.48 and 0.96 kg ai/ha. In 2004, the application timings of S-metolachlor were 46 and 23 days before crop sowing (DBS) and at crop sowing (AS). The herbicide was incorporated by sowing (IBS) for all application dates. In 2005, the application timings of S-metolachlor were 40 and 20 DBS and AS applied either IBS or post sowing pre-emergence (PSPE). The additional application timing [AS (PSPE)] was included in 2005 to compare its crop safety with AS (IBS) application of the herbicide. The total rainfall between the first herbicide application (46 or 40 DBS) and crop sowing was 42 mm in 2004 and 52 mm in 2005. The herbicide was applied using a 5-m wide boom sprayer that delivered 100 L/ha spray solution through flat fan nozzles at a spray pressure of 200 kPa, mounted on a four-wheel motorbike (speed-5 km/h).

The depth of sowing was targeted at 3 cm. The distance between the crop rows was 25 cm and the crop was sown at the seed rate of 90 kg/ha. In both years, urea (100

kg/ha) and diammonium phosphate (100 kg/ha) were drilled with the wheat seed. In 2004, annual ryegrass seed was spread at a rate of 200 seeds/m<sup>2</sup> as the site did not have a seed bank of annual ryegrass. Due to lower establishment of seedlings in 2004, the rate of annual ryegrass seed was increased to 800 seeds/m<sup>2</sup> in 2005. In both years, the area of the experimental plots was 7 m<sup>2</sup> (4.0 by 1.75 m). The experiments were arranged in a split-plot design with application timing as the main plots and dose (0, 0.48 and 0.96 kg/ha) of S-metolachlor as the sub-plots. There were three replicates of each treatment.

#### 7.3.2 Bioavailability of S-metolachlor

A bioassay experiment was conducted in a naturally-lit glasshouse in 2005 to determine the bioavailability of S-metolachlor in the field. The temperature in the glasshouse fluctuated between  $25 \pm 5$  C during the day and  $15 \pm 5$  C during the night. Preliminary experiments were conducted to develop a suitable bioassay technique that was simple to use. Wheat, oat (*Avena sativa* L. cv. Swan), annual ryegrass and canary grass (*Phalaris aquatica* L.) were tested by germinating seeds in pots containing the herbicide ranging from 0 to 0.64 µg/g soil. Assuming a bulk soil density of 1.4 g/cm<sup>3</sup> (Rainbow 2000), the dose of the applied S-metolachlor at 0.48 and 0.96 kg/ha was calculated to be equivalent to 0.32 and 0.64 µg/g soil, respectively.

Wheat was the only species found to be suitable for the bioassay; therefore, inhibition of wheat root growth was selected as the bioassay criterion to study the bioavailability of S-metolachlor. In 2005, the soil samples were taken from the field experiment treated with S-metolachlor at 0.48 and 0.96 kg/ha and from application timings of 20 DBS, AS (IBS) and AS (PSPE). The upper 0 to 5 cm soil layer was sampled from all replications at 0, 8, 14, 23 and 33 days after crop sowing (DAS), taking ten 4.5 cm-diameter cores randomly from each plot. During sampling in the field, the soil samples were placed in sealed plastic bags and stored temporarily in styrofoam boxes containing ice. On returning from the field, the soil samples were frozen at -18 C until analysis.
The frozen soil samples were thawed for 6 hours at room temperature before placing in the pots. The soil sample from each plot was thoroughly mixed and 550 g samples were placed in 600-ml clear-plastic pots. The pots had holes at the bottom and aluminium foil was wrapped around the pots to eliminate light effects on the roots. Concurrently, a standard curve of wheat root response to increasing S-metolachlor dose was developed. Nontreated soil from the same field that had been air-dried was used to prepare standards between 0 and 0.64  $\mu$ g S-metolachlor/g soil.

Six wheat seeds were seeded in each pot at a depth of 0.5 cm and a thin layer of nontreated soil was spread on the surface. The pots were then placed in the glasshouse and arranged in a randomised complete block design with three replications. Wheat roots were washed from the soil 21 days after sowing when seedlings in the nontreated soil had reached the 2.5 to 3-leaf stage. The four most uniform seedlings from each pot were used to measure their root length. Wheat roots were scanned with a flatbed scanner and total root length per plant determined with the WinRHIZO program.

#### 7.3.3 Emergence and Growth of Annual Ryegrass

The efficacy of S-metolachlor on annual ryegrass was evaluated at 30 DAS. Annual ryegrass seedlings were counted in two quadrats (0.5 m by 0.5 m) placed at random in each plot. Herbicide efficacy was calculated by comparing weed density in the S-metolachlor-treated plots with the nontreated plots. Similarly, annual ryegrass spikes were counted at maturity. The dry matter of annual ryegrass plants was taken in 2005. The seed production of annual ryegrass was estimated from the plant samples taken at maturity.

#### 7.3.4 Growth and Grain Yield of Wheat

Various morphological traits of wheat were recorded during the growing season. Wheat seedling emergence under different treatments was determined at 30 DAS. Wheat seedlings were counted in two quadrats (0.5 m by 0.5 m) placed at random in each plot. The leaf area index (LAI) and dry matter of wheat were determined at 30 DAS by randomly sampling 10 plants per plot. A Delta- $T^1$  leaf area meter was used to determine the leaf area and, then the leaf area was converted to the LAI. The plants were placed in paper bags and oven-dried at 65 C for 48 hours for dry weight measurements.

The heads of wheat were counted at maturity by randomly placing two quadrats (0.5 m by 0.5 m) in each plot. Mean number of grains per head was determined by randomly sampling 20 heads per plot. The grain yield of wheat was determined at maturity by harvesting the whole plot with a small-plot harvester. The 1000-grain weight was determined from the harvested grain sample.

#### 7.3.5 Statistical Analyses

All data were subjected to analysis of variance (ANOVA) to evaluate differences and interactions among treatments and the means were distinguished using the LSD test (P = 0.05). Association between morphological traits and grain yield of wheat was examined using simple correlation analysis. Because of an additional application timing treatment in 2005, data were not combined and are presented separately by year. Annual ryegrass density (in both years) and seed production (in 2005) were subjected to the square root transformation. No transformations were found necessary, except for the density and seed production of annual ryegrass.

To determine herbicide bioavailability, an exponential decay curve of the form:

$$y = a \times e^{-bx}$$
 [7.1]

was fitted to the mean root length of wheat plants grown at known herbicide concentrations (standards) by using SigmaPlot 2004 (version 9.0), where y represents the root length at bioavailable concentration x of the herbicide, and a and b are the fitted constants. Bioavailable concentrations of herbicide present in the field soil were estimated by fitting the data for root length (of herbicide-treated soil from the field) to Equation 7.1. The estimated bioavailable concentrations of herbicide were then converted to the percentage of the original applied amount. The data were analysed by two-way ANOVA in a randomised block design with application timing

<sup>&</sup>lt;sup>1</sup> Delta-T Area Meter, Delta-T Devices Ltd., 128 Low Road, Burwell, Cambridge, CB5 OEJ, UK.

and sampling time (DAS) as the two factors. Genstat version 6.0 was used for statistical analysis of all data (Genstat 5 Committee 1993).

### 7.4 Results and Discussion

#### 7.4.1 Bioavailability of S-metolachlor

For weed control it is not the total amount of S-metolachlor that is important, but its bioavailability. Therefore, a bioassay is an effective procedure to determine the bioavailability of an herbicide in the soil (Bunting et al. 2003; Parker et al. 2005; Zimdahl and Clark 1982). In the standard curve, 50% inhibition of wheat root growth occurred between 0.08 and 0.16  $\mu$ g S-metolachlor/g soil (Figure 7.1). The bioassay was conducted on soil sampled from plots treated with both rates (0.48 and 0.96 kg/ha) of S-metolachlor applied in the field in 2005; however, wheat roots were very sensitive in the soils treated with 0.96 kg/ha. Therefore, data are shown only for soil sampled from plots treated with 0.48 kg/ha S-metolachlor. The bioavailable S-metolachlor in the field soil was estimated by fitting the equation to the root length data (Table 7.2). This bioavailable S-metolachlor was then converted to percent bioavailability of the applied herbicide (Figure 7.1).

The concentration of bioavailable S-metolachlor was very similar between the application timings AS (IBS) and AS (PSPE) at both 0 DAS (94 to 96%) and 8 DAS (86 to 89%) (Figure 7.1). After this period, herbicide bioavailability was significantly greater in the AS (IBS) than AS (PSPE). As the herbicide applied AS (PSPE) was not incorporated, a greater portion of the herbicide would have been present on the soil surface and vulnerable to photodegradation losses. The bioavailability of S-metolachlor was always greater for the herbicide applied AS (IBS) than applied at 20 DBS. The bioavailability of the herbicide applied at 20 DBS was 55% of the original applied herbicide at seeding. On the last sampling time (33 DAS) the bioavailability of S-metolachlor was 45, 27 and 28% of the original amount applied for application timings of AS (IBS), AS (PSPE) and 20 DBS, respectively.



Figure 7.1. Exponential relationship in a standard curve between the dose of S-metolachlor (0 to 0.64  $\mu g/g$  soil) and root length of wheat in the bioassay. **Bioassay** was used to determine the bioavailability of S-metolachlor (% of applied) when applied at different times. The field application rate of 0.48 kg/ha S-metolachlor was equivalent to concentration of 0.32  $\mu$ g/g soil. Vertical bars with caps represent standard error.

**Table 7.2.** Wheat root length and bioavailable S-metolachlor determined by bioassay. The applied S-metolachlor concentration was equivalent to  $0.32 \ \mu g/g$  soil. The soil samples were taken at different times after crop sowing (DAS) in 2005.

Time	Wheat root length			Bioavail	able S-metola	achlor
	AS (PSPE)	AS (IBS)	20 DBS	AS (PSPE)	AS (IBS)	20 DBS
DAS		-cm/plant		h	ug/g of soil—	
0	36.6	37.7	64.7	0.31	0.30	0.18
8	40.6	41.7	68.8	0.28	0.28	0.16
14	62.8	53.4	73.0	0.18	0.22	0.15
23	87.0	58.0	77.8	0.11	0.20	0.14
33	96.3	75.1	94.4	0.09	0.14	0.09

Lower amount of bioavailable S-metolachlor in the 20 DBS treatment at the time of sowing (0 DAS) could be due to photodegradation losses occurring since its

application (e.g. Mathew and Khan 1996). Photodegradation could be a major contributor to dissipation in the field, particularly under prolonged lack of rainfall when the herbicide remains on the soil surface rather than being incorporated by rainfall.

### 7.4.2 Emergence and Growth of Annual Ryegrass

In both years, annual ryegrass density was significantly influenced by the interaction between application timing and herbicide (Table 7.3). In the absence of herbicide application, annual ryegrass density was greater in 2005 than in 2004. In spite of greater annual ryegrass density, overall percent control from the herbicide was similar between the years. In general, the control provided by S-metolachlor at 0.48 and 0.96 kg/ha was similar.

**Table 7.3.** Effects of application timing and dose of S-metolachlor on density of annual ryegrass in 2004 and 2005. Data were subjected to square root transformation before analysis. Values in parentheses represent original values.

Application	Do	ose of S-metolach	lor	LSD <sup>a</sup> 0.05
timing	Nontreated	0.48 kg/ha	0.96 kg/ha	
		plants/m <sup>2</sup>		
		2004		
AS (IBS)	7.09 (50.0)	3.14 (9.4)	2.37 (5.2)	
23 DBS	6.58 (43.3)	3.51 (11.4)	2.78 (7.3)	0.95
46 DBS	6.65 (44.0)	5.49 (29.7)	5.28 (27.4)	
LSD <sup>b</sup> 0.05		0.78		
		2005		
AS (IBS)	18.81 (363.3)	8.13 (66.7)	7.48 (56.7)	
AS (PSPE)	19.97 (400.0)	8.34 (70.0)	7.95 (63.3)	2.02
20 DBS	19.76 (390.0)	8.77 (80.0)	8.13 (66.7)	3.23
40 DBS	18.30 (336.7)	14.56 (213.3)	13.18 (173.3)	
LSD <sup>b</sup> 0.05		2.96		

<sup>a</sup>LSD for comparing means within an application timing.

<sup>b</sup>LSD for comparing means within a herbicide dose.

In both years, the control of annual ryegrass was greater when the herbicide was applied from sowing to 23 DBS (74 to 90%) than when applied 40 or 46 DBS (33 to 49%) (Table 7.3). Despite the bioavailable S-metolachlor declining by 45% between application 20 DBS and seeding in 2005, the control of annual ryegrass density was still 79%. This suggests the possibility that S-metolachlor may be absorbed into seeds of annual ryegrass during the pre-seeding period. The reduced control with S-metolachlor applied 40 DBS is likely due to even greater dissipation of the herbicide on the surface of the soil over this extended dry period. During the first 20 days after the first herbicide application (40 or 46 DBS), there was only 11 mm and 2 mm rainfall in 2004 and 2005, respectively. This extended dry period after herbicide application may have resulted in photodecomposition of this herbicide.

S-metolachlor normally suffers significant losses due to photodegradation under extended dry conditions (Ahrens 1994). However, in a glasshouse study the efficacy of S-metolachlor applied to the soil surface was not reduced by exposure to increasing periods of high temperature, intense sunlight, and/or very dry conditions (Walsh et al. 2004). In their study, S-metolachlor applied at 0.72 kg/ha provided more than 70% control of annual ryegrass even 84 days after application. In the glasshouse, roof materials filter ultraviolet light, which reduces the potential for photodecomposition losses. Moreover, Walsh et al. (2004) used potting mix containing a high level of organic matter, which might have contributed to the increased retention of S-metolachlor.

Although an interaction was observed between application timing and dose of herbicide for the density of annual ryegrass, it was not observed for the number of spikes, dry matter and seed production of annual ryegrass. These traits were influenced by the main effects of application timing in 2004 and dose of herbicide in both years (Tables 7.4 and 7.5).

In 2004, the number of spikes and seed production of annual ryegrass were lowest when the herbicide was applied AS (IBS) (Table 7.4). However, there were no significant differences in these traits in 2005 (Table 7.5). In the absence of herbicide application, annual ryegrass produced 1572 seeds/m<sup>2</sup> in 2004 and 8717 seeds/m<sup>2</sup> in

2005. The control of annual ryegrass seed set by S-metolachlor was 70 to 79% and 46 to 58% in 2004 and 2005, respectively.

Although the density of annual ryegrass was controlled effectively by some of the treatments, the surviving plants still produced a significant amount of seed. Therefore, S-metolachlor will need to be combined with integrated weed management practices that prevent replenishment of weed seed bank. Such practices include use of non-selective herbicides (e.g. glyphosate) to prevent seed production or use of weed seed catchers during the harvest operation.

**Table 7.4.** Effects of application timing and dose of S-metolachlor on annual ryegrass spike and seed production in 2004.

Main effect	Spikes	Seed production
	1	no/m²
Application timing		
AS (IBS)	29.6	686
23 DBS	34.7	811
46 DBS	36.9	867
LSD 0.05	—4.5—	—78—
Dose of S-metolachlor		
Nontreated	70.4	1572
0.48 kg/ha	18.2	467
0.96 kg/ha	12.4	325
LSD 0.05	—5.4—	—100—

### 7.4.3 Growth and Grain Yield of Wheat

The seedling emergence of wheat was significantly influenced by the interaction between application timing and herbicide dose (Table 7.6). In both years, the seedling emergence of wheat was similar between the three doses when applied at 20 to 23 DBS and 40 to 46 DBS. On the other hand, emergence was reduced at both rates when herbicide was applied at sowing [AS (IBS) or AS (PSPE)] compared to the nontreated control. In 2004, when herbicide was applied AS (IBS) wheat

emergence was 14 and 24% lower at 0.48 and 0.96 kg/ha compared to the nontreated control. At this application timing in 2005, emergence was 44 and 58% at 0.48 and 0.96 kg/ha, respectively, compared to the nontreated. The reduction in wheat emergence was even greater when the herbicide was applied AS (PSPE).

**Table 7.5.** Effects of application timing and dose of S-metolachlor on annual ryegrass spike number, dry matter and seed production in 2005. Seed production data was subjected to square root transformation before analysis. Values in parentheses represent original values.

Main effect	Spikes	Dry matter	Seed production
	no/m²	<u> </u>	no/m²
Application timing			
AS (IBS)	148	62.4	62.1 (5004)
AS (PSPE)	176	76.3	77.3 (6651)
20 DBS	160	65.0	66.4 (5419)
40 DBS	177	84.4	72.1 (5661)
LSD 0.05	—NS—	—NS—	—NS—
Dogo of S motol apploy			
Dose of S-metolachior			
Nontreated	281	121.5	92.4 (8717)
0.48 kg/ha	139	57.5	61.5 (4707)
0.96 kg/ha	76	37.1	54.5 (3629)
LSD 0.05	—74—	—36.2—	—24.0—

Greater herbicide phytotoxicity on wheat seedlings in 2005 than in 2004 could be due to environmental conditions (Table 6.2) or soil properties (e.g. organic matter content). The organic matter content was greater in 2004 (3.4%) compared to 2005 (2.6%). Soils having high organic matter are expected to have greater microbial activity and this herbicide is mainly degraded by microbes (Beestman and Deming 1974; Zimdahl and Clark 1982). Furthermore, soils having less organic matter are expected to have less adsorption and greater herbicide bioavailability. In addition, soil texture (e.g. clay content) could also affect the activity of the herbicide. Rainfall was greater after crop sowing in 2005 compared to 2004, which might have caused the herbicide to move into the furrows and increased exposure of wheat to S-metolachlor.

Application	Do	ose of S-metolach	lor	LSD <sup>a</sup> 0.05
timing	Nontreated	0.48 kg/ha	0.96 kg/ha	•
		plants/m <sup>2</sup>		
		2004		
AS (IBS)	263.5	227.6	200.5	
23 DBS	275.0	265.4	254.2	25.9
46 DBS	271.9	266.7	268.8	
LSD <sup>b</sup> 0.05		25.4		
		2005		
AS (IBS)	268.0	150.7	112.0	
AS (PSPE)	265.3	110.7	64.0	26.0
20 DBS	269.3	250.7	242.7	30.2
40 DBS	273.3	269.3	265.3	
LSD <sup>b</sup> 0.05		60.6		

**Table 7.6.** Effects of application timing and S-metolachlor dose on wheat emergenceat 30 days after sowing in 2004 and 2005.

<sup>a</sup>LSD for comparing means within an application timing.

<sup>b</sup>LSD for comparing means within a herbicide dose.

The LAI and dry matter of wheat at 30 DAS were influenced by the interaction between application timing and dose of herbicide (Table 7.7 and 7.8). The interactions observed were mainly due to the greater LAI and dry matter of wheat in the nontreated plots compared to the herbicide-treated plots when the herbicide was applied at crop sowing. Across the herbicide-treated plots, the LAI and dry matter of wheat were significantly different (P > 0.05) at different application timing of the herbicide, which may have also contributed to the interactions observed.

In 2004, the LAI of wheat was similar between the dose treatments when Smetolachlor was applied at 23 and 46 DBS (Table 7.7). On the other hand, a reduction of LAI was observed at both herbicide rates when the herbicide was applied AS (IBS). The LAI was 34 and 48% lower at 0.48 and 0.96 kg/ha S-metolachlor compared to the untreated plots. At this application timing in 2005, the reduction in the LAI was 54 and 70% at 0.48 and 0.96 kg/ha S-metolachlor, respectively. The reduction in the LAI was even greater (70 to 86%) when the herbicide was applied AS (PSPE).

**Table 7.7.** Effects of application timing and dose of S-metolachlor on leaf area index of wheat at 30 days after sowing in 2004 and 2005.

Application	Do	ose of S-metolach	lor	LSD <sup>a</sup> 0.05
timing	Nontreated	0.48 kg/ha	0.96 kg/ha	-
		2004		
AS (IBS)	0.134	0.088	0.070	
23 DBS	0.133	0.129	0.108	0.025
46 DBS	0.121	0.136	0.129	
LSD <sup>b</sup> 0.05		0.035		
		2005		
AS (IBS)	0.132	0.061	0.040	
AS (PSPE)	0.132	0.039	0.018	0.018
20 DBS	0.125	0.101	0.089	0.018
40 DBS	0.149	0.149	0.122	
LSD <sup>b</sup> 0.05		0.039		

<sup>a</sup>LSD for comparing means within a application timing.

<sup>b</sup>LSD for comparing means within a herbicide dose.

Similar to the LAI, the dry matter of wheat (at 30 DAS) in 2004 was 32 and 42% lower at 0.48 and 0.96 kg/ha S-metolachlor when the herbicide was applied AS (IBS) compared to the control (Table 7.8). At this application timing in 2005, the reduction in dry matter was 51 and 68% at 0.48 and 0.96 kg/ha S-metolachlor, respectively. The reduction in dry matter was even greater (65 to 83%) when the herbicide (0.48 and 0.96 kg/ha) was applied AS (PSPE).

Application	Do	ose of S-metolach	lor	LSD <sup>a</sup> 0.05
timing	Nontreated	0.48 kg/ha	0.96 kg/ha	
		g/m²		
		2004		
AS (IBS)	9.8	6.7	5.7	
23 DBS	9.7	9.0	8.4	1.9
46 DBS	9.0	10.2	10.3	
LSD <sup>b</sup> 0.05		3.0		
		2005		
		2003		
AS (IBS)	7.8	3.8	2.5	
AS (PSPE)	7.7	2.7	1.3	17
20 DBS	7.9	6.8	5.9	1.7
40 DBS	9.1	9.6	7.6	
$LSD^{b} 0.05$		2.6		

**Table 7.8.** Effects of application timing and dose of S-metolachlor on dry matter of wheat at 30 days after sowing in 2004 and 2005.

<sup>a</sup>LSD for comparing means within an application timing.

<sup>b</sup>LSD for comparing means within a herbicide dose.

In both years, the density of wheat heads was significantly influenced by the interaction between application timing and dose of S-metolachlor (Table 7.9). The interactions observed could be due to the lower number of heads in the S-metolachlor-treated plots, particularly at 0.96 kg/ha, compared to the nontreated plots when the herbicide was applied at crop sowing.

In 2004, the number of wheat heads was not influenced by the dose of S-metolachlor when applied at 23 or 46 DBS (Table 7.9). However, there was a 6 to 12% reduction in the number of wheat heads when the herbicide was applied AS (IBS). In 2005 when the herbicide was applied at AS (IBS), the wheat head density was similar between the nontreated and 0.48 kg/ha S-metolachlor-treated plots, but there was a 22% reduction in the head density observed in the 0.96 kg/ha S-metolachlor-treated plots. The reduction in head density was even greater (14 to 38%) when herbicide

was applied AS (PSPE). This reduction was due to the high phytotoxic effect observed on the seedling emergence of wheat.

Application	Do	ose of S-metolach	lor	LSD <sup>a</sup> 0.05
timing	Nontreated	0.48 kg/ha	0.96 kg/ha	-
		no/m²		
		2004		
AS (IBS)	375	354	329	
23 DBS	371	365	358	12
46 DBS	358	355	347	
LSD <sup>b</sup> 0.05		32		
		2005		
AS (IBS)	287	284	223	
AS (PSPE)	287	246	177	20
20 DBS	281	338	341	39
40 DBS	289	301	313	
LSD <sup>b</sup> 0.05		38		

**Table 7.9.** Effects of application timing and dose of S-metolachlor on density of wheat heads in 2004 and 2005.

<sup>a</sup>LSD for comparing means within an application timing.

<sup>b</sup>LSD for comparing means within a herbicide dose.

In 2005, due to the greater plant density of annual ryegrass in the nontreated plots, a 17 to 18% increase in wheat head density was observed when S-metolachlor was applied at 20 DBS. The increase in wheat head density was very small (4 to 7%) when the herbicide was applied 40 DBS (Table 7.9). This could be due to the greater competition of wheat with annual ryegrass in the plots treated with the herbicide at 40 DBS (Table 7.3).

Although an interaction was observed between application timing and dose of herbicide for the density of wheat heads, it was not observed for the head size (grains/head) and seed size (1000-grain weight). The number of grains/head was

influenced only by application timing in 2004 (Table 7.10 and 7.11). The number of grains/head was greater when the herbicide was applied AS (IBS) than 46 DBS. The lower competition between the wheat plants growing at a lower density in this treatment could have contributed to the greater number of wheat grains/head. In both years, the size of wheat grain was lower in the herbicide-treated plots than in the nontreated plots (Table 7.10 and 7.11).

Main effect Grains/head 1000-grain weight —no— —g— Application timing AS (IBS) 43.4 32.18 **23 DBS** 41.4 32.43 46 DBS 40.4 32.04 LSD 0.05 -2.0-\_\_NS\_\_ Dose of S-metolachlor Nontreated 41.8 32.95 0.48 kg/ha 40.8 32.02 42.5 0.96 kg/ha 31.68 LSD 0.05 —NS— -0.43-

**Table 7.10.** Effects of application timing and dose of S-metolachlor on grains/head and 1000-grain weight of wheat in 2004.

The grain yield of wheat was significantly influenced by the interaction between application timing and dose of herbicide (Table 7.12). Grain yield was reduced when the herbicide was applied AS (IBS) and AS (PSPE) compared to the nontreated control. This was due to reduced wheat emergence, a phytotoxic response to the herbicide. Ritter and Menbere (2002) also observed minor stunting of wheat plants where high rates of S-metolachlor (0.56 to 1.12 kg/ha) were used; however, symptoms were transient and grain yields were not affected.

In 2004, no negative effect of S-metolachlor application at either 23 or 46 DBS was found on wheat grain yield. The low density of annual ryegrass plants in the nontreated plots (Table 7.3) was unlikely to have provided significant competition to wheat. In 2005, due to the greater plant density of annual ryegrass, an increase in grain yield was observed with S-metolachlor applied at 20 DBS, but not at any other timing. This treatment not only provided effective control of annual ryegrass, it was safe to wheat. In 2005, application of S-metolachlor AS (PSPE) significantly decreased grain yield at both herbicide doses. When the herbicide was applied at 40 or 46 DBS, it was not phytotoxic to wheat but failed to control annual ryegrass effectively.

Main effect	Grains/head	1000-grain weight
	—no—	g
Application timing		
AS (IBS)	34.0	25.76
AS (PSPE)	34.2	25.18
20 DBS	34.7	26.60
40 DBS	35.0	26.68
LSD 0.05	—NS—	—NS—
Dose of S-metolachlor		
Nontreated	34.5	27.03
0.48 kg/ha	34.5	25.92
0.96 kg/ha	34.4	25.21
LSD 0.05	—NS—	0.64

**Table 7.11.** Effects of application timing and dose of S-metolachlor on grains/head

 and 1000-grain weight of wheat in 2005.

In both years, the response of grain yield was positively correlated with the seedling emergence, LAI, dry matter and head density of wheat (Table 7.13). Head density of wheat is known to be a useful indicator of the potential crop yield. In our study, head density was strongly correlated (P < 0.01) with grain yield. In 2005, the grain yield was positively correlated with the 1000-grain weight, which also might have contributed in the increase in grain yield in the plots where the competition from annual ryegrass was low.

Application	Do	ose of S-metolach	lor	LSD <sup>a</sup> 0.05	
timing	Nontreated	0.48 kg/ha	0.96 kg/ha		
		kg/ha			
		2004			
AS (IBS)	3362	3148	2947		
23 DBS	3332	3302	3218	170	
46 DBS	3184	3166	3147		
LSD <sup>b</sup> 0.05		224			
		2005			
AS (IBS)	2599	2327	1871		
AS (PSPE)	2475	2062	1358	202	
20 DBS	2505	3146	3132	302	
40 DBS	2544	2640	2650		
LSD <sup>b</sup> 0.05		346			

Table 7.12. Effects of appl	ication timing and do	ose of S-metolachlor or	grain yield of
wheat in 2004 and 2005.			

<sup>a</sup>LSD for comparing means within an application timing.

<sup>b</sup>LSD for comparing means within a herbicide dose.

Table	7.13.	Correlation	coefficient	between	morphological	traits	and	grain	yield
(kg/ha	) of wl	heat.							

Trait	Grain yield	
-	2004	2005
Wheat emergence (plants/m <sup>2</sup> )	0.52**	0.78**
LAI at 30 DAS	0.55**	0.70**
Wheat dry matter (g/m <sup>2</sup> ) at 30 DAS	0.45*	0.73**
Head density (number/m <sup>2</sup> )	0.95**	0.91**
Grains/head	0.12	0.13
1000-grain weight (g)	0.25	0.75**

\**P*<0.05; \*\**P*<0.01.

## 7.5 Conclusions

This field study has identified the potential for a novel approach to control annual ryegrass in the current farming systems of southern Australia. The study indicates that S-metolachlor at 0.48 kg/ha could be safely applied around 20 DBS to selectively control annual ryegrass in wheat. To prevent increase in annual ryegrass infestation, use of S-metolachlor needs to be integrated with other management practices that prevent seed production by surviving plants. Very early application (40 DBS or more) may decrease the efficacy of S-metolachlor in controlling annual ryegrass. Pre-seeding application of S-metolachlor has the potential to increase the speed of crop seeding operations, as there may not be a requirement to apply an herbicide at seeding. Above all, it may provide a means of effectively controlling herbicide-resistant annual ryegrass. Incorporation of this herbicide by pre-seeding tillage could reduce herbicide degradation and increase the control of annual ryegrass if applied very early (40 DBS or more). However, such tillage could also increase the risk of crop phytotoxicity by reducing the separation between the crop seed and the herbicide. Further research will be required on other soil types and climatic conditions to demonstrate the robustness of early S-metolachlor application as a viable strategy for managing annual ryegrass in wheat crops.

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# **Chapter 8. General discussion**

The use of reduced tillage systems has been rapidly increasing in Australia over the past decade and this trend is expected to continue over the next few years (D'Emden and Llewellyn 2004). These changes in tillage practices can have a major influence on the ecology and management of weeds. Therefore, it is important to understand the mechanisms responsible for the changes in weed behaviour under changing tillage systems. Research presented in this thesis provides important new information on the influence of tillage systems on the ecology and management of weeds in southern Australia.

## 8.1 Tillage Effects on Weed Ecology

Studies were undertaken on the seed biology of important Australian weed species to provide underpinning knowledge of their response to tillage (Chapter 3). Weed species were found to differ in their germination responses to light. Light stimulated seed germination in some weed species [e.g. common sowthistle (*Sonchus oleraceus* L.) and Indian hedge mustard (*Sisymbrium orientale* L.)] and inhibited germination in others [e.g. threehorn bedstraw (*Galium tricornutum* Dandy) and wild turnip (*Brassica tournefortii* Gouan), the latter only at sub-optimal temperatures]. Species showing preference for light in germination have the potential to be more prevalent in no-till (NT) systems (Cousens et al. 1993), as these systems tend to concentrate weed seeds on or close to the soil surface (Pareja et al. 1985; Yenish et al. 1992, 1996). Similar patterns of vertical seed distribution by tillage systems were also found in this study (Chapter 5). Therefore, species such as common sowthistle and Indian hedge mustard are expected to experience greater seedling emergence and may become more abundant under low soil disturbance systems (e.g. MINT).

On the other hand, species with preference for darkness for germination are expected to show greater seedling emergence under high soil disturbance systems compared to low soil disturbance systems. Under NT systems, such species are likely to establish only after crop sowing which may reduce their competitive ability with the crop. On the other hand, it may become more difficult to control these species with pre-sowing application of non-selective herbicides.

Seedling emergence of weed species such as common sowthistle and Indian hedge mustard was greatest for the seeds present on the soil surface and emergence decreased with increased burial depth. These results are consistent with the stimulation of germination in the presence of light, as seeds present on the soil surface receive a considerable amount of light. On the other hand, seeds buried more than 2 mm below the soil surface usually receive less than 1% of incident light (Egley 1986; Woolley and Stoller 1978). The results again suggest that these weed species have the potential of becoming problematic weeds under NT systems, in which a large proportion of the seed bank remains on the soil surface after crop planting.

Seedling emergence of other weed species such as threehorn bedstraw, wild turnip, small-flowered mallow (*Malva parviflora* L.) and annual ryegrass (*Lolium rigidum* Gaud.) was low on the soil surface but increased with shallow burial, which suggests that farming practices that achieve shallow burial of seeds are likely to promote greater seedling emergence of these weed species. Light is not inhibitory for seed germination in annual ryegrass (Steadman et al. 2003) and small-flowered mallow (Chapter 3), so lower seedling emergence of these species on the soil surface was probably due to the lower availability of moisture.

In the literature, weed seedling emergence responses to tillage have been shown to be species-specific (e.g. Buhler and Daniel 1988). Similar species-specific responses were reported in this thesis when comparative studies were undertaken with NT and MINT systems (Chapter 4). Seedling emergence of threehorn bedstraw and wild radish (*Raphanus raphanistrum* L.) was greater under MINT than NT system. The greater seedling emergence of these weed species under MINT might be due to stimulation of germination in these species by darkness (Piggin et al. 1978 and Chapter 3). The seedling emergence of closely related catchweed bedstraw (*G. aparine* L.) and false cleavers (*G. spurium* L.) was also stimulated by tillage in Canada (Reid and Van Acker 2005).

In contrast, the seedling emergence of light responsive species, such as common sowthistle and Indian hedge mustard was greater under NT compared to MINT. Greater seedling emergence of common sowthistle, Indian hedge mustard and silvergrass (*Vulpia bromoides* (L.) S.F. Gray) under NT system could also be related to their small seed size (Chapter 4), which failed to emerge when buried deeply by MINT. Seedling emergence of silvergrass may be severely reduced at depths greater than 10 mm (Dillon and Forcella 1984) and, therefore, adoption of direct-drill techniques may be responsible for its increasing prominence as a weed in cereal crops (Amor and de Jong 1983).

Although seed germination of small-flowered mallow was not influenced by light, the seedling emergence was greater under NT compared to MINT. This observed response could be due to greater scarification of seeds by more variable environmental conditions on the soil surface, as scarification was found to stimulate germination in this species (Chorbadjian and Kogan 2004 and Chapter 3). In the field, natural scarification is usually brought about by means such as trampling by animals, incomplete predation by insects or rodents, damage by fungi or soil microorganisms, passage through the digestive tract of animals, tillage implements and extreme changes in temperatures (e.g. Taylor 2005). Under NT systems of cropping, scarification due to tillage would only occur at sowing. Consequently, small-flowered mallow would only emerge after crops have been sown thus making it more difficult to control. Anecdotal evidence supports the greater densities of small-flowered mallow under NT. However, this response could also be due to tolerance to glyphosate (a non-selective herbicide), which is being widely used under NT systems. Seed germination of other weed species, such as wild oat (Avena fatua L.) and wild turnip, was not influenced by the tillage systems.

Light stimulates seed germination in annual ryegrass (Steadman et al. 2003), but the seedling emergence of annual ryegrass was lower under NT compared with MINT. This response could be due to rapid desiccation of seeds present on the soil surface under NT system (Mohler and Galford 1997). In contrast, MINT adequately covered the weed seed with soil and provided more favourable moisture and temperature conditions for germination and seedling establishment.

Detailed studies were conducted to study the tillage effects on seedling emergence and persistence of annual ryegrass seed bank (Chapter 5), which showed a large inhibition in seedling emergence under low soil disturbance systems (e.g. NT and discs) compared with the high soil disturbance systems (e.g. MINT and tines). For example, seedling emergence of annual ryegrass was 2 to 4-fold greater under MINT than under NT. The start of seedling emergence was also delayed under low soil disturbance systems compared with high soil disturbance systems. Consequently, the tillage systems that caused the lowest soil disturbance resulted in less vigorous annual ryegrass plants. Slower seedling emergence under NT could have implications for the management of this species in the field. It could mean that late emerging plants may be less competitive with the crop and not have a substantial impact on crop yield loss and weed seed production (O'Donovan et al. 1985).

Although high and low soil disturbance tillage systems had large effects on the seedling emergence of annual ryegrass, they did not influence the persistence of the annual ryegrass seed bank. Irrespective of the tillage system, seed bank persistence was found to range from 7 to 14% at the Roseworthy Campus and 16 to 30% at Minlaton. This consistency in seed bank persistence across the tillage systems was due to the compensatory effect of greater seed decay under the low soil disturbance systems. For example, seed decay under NT (48 to 60%) was much greater than MINT (12 to 39%). Greater levels of seed decay under the low soil disturbance systems could result from an unfavourable microenvironment for seedling emergence on the soil surface or because of greater predation activity of insects on or near the soil surface (Mohler and Galford 1997; Taylorson 1970).

The results presented in this thesis suggest that the influence of tillage systems on weed seedling emergence is species-specific. Small-seeded species often require light for germination and mainly emerge from the surface layer; consequently these species have a greater potential to emerge effectively under NT systems. In contrast, most of the large-seeded weed species were favoured by MINT, which could be due to inhibition of germination by light or lower dependency on light for germination as well as greater energy reserves for emergence from deeper depths. The information on the effect of tillage systems on the seedling emergence of different weed species will be useful for developing prediction models. Such information could also be useful in deciding optimal timing for the control of weeds in crops. The study also found greater seed decay of annual ryegrass under low soil disturbance NT systems compared to the high soil disturbance systems. This indicates that the adoption of NT is unlikely to aggravate the problem of annual ryegrass, thus giving confidence to extension agencies promoting such systems.

## 8.2 Tillage Effects on Herbicide Activity

Tillage systems can affect efficacy and persistence of herbicides, particularly soilactive herbicides. As NT systems cause low soil disturbance (see Chapter 5), it is likely that a large proportion of the soil-applied (pre-sowing) herbicides remains on the soil surface after crop planting. Depending on the herbicide chemistry, this proportion is vulnerable to volatilisation and photodegradation losses. For example, trifluralin, which has a high vapour pressure, is thought to dissipate mainly by photodecomposition and volatilisation (Grover et al. 1997).

The studies reported in this thesis have clearly demonstrated that trifluralin bioavailability was greater under tillage systems that provided higher levels of soil disturbance than the lower soil disturbance systems (Chapter 6). Less herbicide loss under the high soil disturbance tillage systems is likely due to greater soil coverage of the herbicide, as incorporation of trifluralin has been shown to increase its persistence in the soil (Savage and Barrentine 1969). Under low soil disturbance systems, most of the applied herbicide might have remained on the soil surface even after the sowing operation, where it will be susceptible to loss through volatilisation and photodecomposition.

The efficacy of different dinitroaniline herbicides, especially trifluralin on annual ryegrass was lower in the low soil disturbance systems compared to the high soil disturbance systems. For example at the Roseworthy Campus, trifluralin and pendimethalin provided 61 to 80% and 44 to 57% control of annual ryegrass under MINT and NT, respectively. This response could be due to the greater loss of herbicides under low soil disturbance systems compared with the high soil disturbance systems. However, the lower efficacy was partly compensated by the lower annual ryegrass seedling emergence under the low soil disturbance tillage

systems compared to the high soil disturbance tillage systems (see section 8.1 above). The seed production of annual ryegrass was also lower in the low soil disturbance systems compared to the high soil disturbance systems. The benefits of lower seed set under these systems could be fully exploited if effective herbicides, which are suitable for use in these systems, can be identified.

In addition to the efficacy and herbicide bioavailability issues, the development of resistance in annual ryegrass against widely used trifluralin warrants further research to find an effective herbicide with a different mode of action (McAlister et al. 1995). NT system offers opportunities to use other herbicides safely by sowing the crop below the herbicide band, provided the herbicide has limited soil mobility. At present there is an enormous interest in using S-metolachlor, which has a different mode of action, for the control of annual ryegrass in cereal crops. Research undertaken in this project has identified the potential for a novel approach to use S-metolachlor for the selective control of annual ryegrass in wheat (*Triticum aestivum* L.) under NT.

This study found that S-metolachlor could be safely applied at 0.48 kg/ha about 20 days before crop sowing (DBS) to selectively control annual ryegrass in wheat. Although the herbicide provided more than 70% control of annual ryegrass plants, the surviving plants were able to produce a considerable amount of seed. Therefore, this herbicide would need to be used in conjunction with other weed management practices that prevent seed production by surviving plants. Such practices include use of non-selective herbicides for 'crop-topping' or use of weed seed catchers during the harvest operation. The very early application (40 DBS or more) of S-metolachlor had much lower efficacy on annual ryegrass, which was likely caused by rapid dissipation of the herbicide under high temperature and dry conditions over a long period of time (Ahrens 1994). Although S-metolachlor applied at crop sowing provided an effective control of annual ryegrass, it was phytotoxic to wheat and reduced its growth and grain yield.

### **8.3 Future Research**

The research undertaken showed that even a moderate level of non-wetting in the soil could affect the persistence of the weed seed bank. Therefore, further research is

needed to determine persistence and seed decay levels in soils with greater levels of non-wetting. Because the effects of tillage systems on seedling emergence and persistence of seed bank could be species specific, research on seed bank persistence is needed for other important cropping weeds of Australia (e.g. threehorn bedstraw, small-flowered mallow, Indian hedge mustard etc.). Weed ecotypes often vary in dormancy characteristics and germination requirements; therefore, inferences drawn from the seed germination study should be limited to the weed populations sampled. Further research is needed to determine the effect of different factors on weed seed germination of various populations collected from different sites.

Further research is also needed to determine the consistency in the performance of early pre-sowing application of S-metolachlor on annual ryegrass control in wheat on different soil types and under different climatic conditions. Research is also needed on the performance of S-metolachlor under low soil disturbance disc systems. Due to its lower vapour pressure and low risk of volatilisation loss, S-metolachlor may be better suited to disc systems than is trifluralin.

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