Weed Resistance Risk Management in Glyphosate-Resistant Cotton

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ABSTRACT

The introduction of glyphosate resistance into Australian cotton systems will have an effect on conventional weed management practices, the weed species present and the risk of glyphosate resistance evolving in weed species. Therefore, it is important that the effects of these management practices, particularly a potential reduction in Integrated Weed Management (IWM) practices, be examined to determine their impact on weed population dynamics and resistance selection.

The study began in 2003 with a survey of 40 growers in four major cotton growing regions in Australia to gain an understanding of how adoption of glyphosate resistance had influenced the weed spectrum, weed management practices and herbicide use after three years of glyphosate-resistant cotton being available. The 10 most common weeds reported on cotton fields were the same in glyphosate-resistant and conventional fields. In this survey, herbicide use patterns were altered by the adoption of glyphosate-resistant cotton with up to six times more glyphosate being applied and with 21% fewer growers applying pre-emergence herbicides in glyphosate-resistant cotton fields. Other weed control practices, such as the use of post-emergence herbicides, inter-row cultivation and hand hoeing, were only reduced marginally.

A systems experiment was conducted to determine differences in the population dynamics of *Echinochloa crus-galli* (barnyardgrass) and *Urochloa panicoides* (liverseed grass) under a range of weed management regimes in a glyphosate-resistant cotton system. These treatments ranged from a full IWM system to a system based soley on the use of glyphosate. The experiment investigated the effect of the treatments on the soil seed bank, weed germination patterns and weed numbers in the field. All applied treatments resulted in commercially acceptable control of the two grass weeds. However, the treatments containing soil-applied residual herbicides proved to be more effective over the period of the experiment. The treatment with a reduced residual herbicide program supplemented with glyphosate had a level of control similar to the full IWM treatments with less input, providing a more economical option. The effectiveness of these treatments in the long-term was examined in a simulation model to determine the likelihood of glyphosate resistance evolving using barnyardgrass and liverseed grass as model weeds.

Seed production and above-ground biomass of barnyardgrass and liverseed grass in competition with cotton were measured. In all experiments, seed production and biomass plant^{-1} decreased as weed density increased while seed production and biomass m^{-1} tended to increase. Seed production m^{-1} reached 40,000 and 60,000 for barnyardgrass and liverseed grass, respectively. In 2004-05, weeds were also planted 6 weeks and 12 weeks after the cotton was planted. Biomass and seed production of the two weeds planted 6 weeks after cotton were significantly reduced with seed production declining to 12,000 and 2,500 seeds m^{-1} row for barnyardgrass and liverseed grass, respectively. Weeds planted 12 weeks after cotton planting failed to emerge. This experiment highlighted the importance of early season weed control and effective management of weeds that are able to produce high seed numbers.

A glyphosate dose-mortality experiment was conducted in the field to determine levels of control of barnyardgrass and liverseed grass. Glyphosate provided effective control of both species with over 85% control when the rate applied was greater than 690 g ae ha^{-1}. Dose-mortality curves for both species were obtained for use in the glyphosate resistance model.

Data from the experimental work were combined to develop a glyphosate resistance model. Outputs from this model suggest that if glyphosate were used as the only form of weed control, resistance in weeds is likely to eventuate after 12 to 17 years, depending on the characteristics of the weed species, initial resistance gene frequencies and any associated fitness penalties. If glyphosate was used in conjunction with one other weed control method, resistance was delayed but not prevented. The simulations suggested that when a combination of weed control options was employed in addition to glyphosate, resistance would not evolve over the 30-year period of the simulation. These simulations underline the importance of an integrated strategy in weed management to prevent glyphosate resistance evolving from the use of glyphosate-resistant cotton. Current management conditions of growing glyphosate-resistant (Roundup Ready®) cotton should therefore prevent glyphosate resistance evolution.

Declaration of Originality

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 any other 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 Library, being made available in all forms of media, now or hereafter known.

Jeff Werth

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CHAPTER 1

INTRODUCTION

The introduction of herbicide-resistant crops represents the next revolutionary breakthrough in weed management (Baldwin 1999). Since their introduction, there has been a rapid increase in the area of herbicide-resistant crops (HRCs) grown around the world despite continuing debate over genetically modified organisms (Malik *et al*. 1989; Baldwin 1999). In Australia, cotton is the first broad acre crop to have genetically modified herbicide tolerant varieties available, being genetically modified to tolerate the herbicide glyphosate (Roberts 1999). As with other HRCs around the world, glyphosate-resistant cotton, developed by Monsanto and sold as Roundup Ready® cotton has had rapid adoption since its introduction in 2000.

Introduction of HRCs, such as Roundup Ready® cotton, poses the same risks as the introduction of a new herbicide that has the same mode of action as herbicides already in use in a production system (Wyse 1991). This raises concerns with regard to weed species shifts and herbicide resistance (Wyse 1991; Lyon 2001; Derksen *et al*. 1999; Forcella 1999). Herbicide-resistant weeds threaten the success of herbicides to contribute effectively to weed management (Powles *et al*. 1997). Herbicide resistance is a consequence of the extensive use of herbicides (Preston and Powles 2002), and in particular, where one of few herbicides has been used persistently in weed management (Preston and Reiger 2000).

Glyphosate resistance in cotton is likely to reduce the number of conventional weed management practices employed, alter the weed species present, and increase the risk of glyphosate resistance evolution. Although there are no reported cases of glyphosate resistance in Australian cotton fields, the question remains: will glyphosate resistance in cotton lead to glyphosate-resistant weed problems for the Australian cotton industry (Roberts 1999)? Due to its mode of action, glyphosate is one of the least likely of all chemicals to have weed species evolve resistance to it, however evolution of glyphosate resistance in seven weed species around the world proves that weeds can evolve resistance to all chemicals when they are used repeatedly (Roberts 1998*a*; Preston *et al*. 1999).

Integrated weed management strategies have prevented the evolution of resistance in weeds of cotton in Australia thus far. Monsanto Australia, in conjunction with weed scientists in the cotton industry, developed a crop management plan in order to discourage a glyphosate-only approach to weed management in glyphosate-resistant cotton in Australia. This plan encourages the use of as many weed control options as possible and stipulates that external auditors assess the incidence of weed escapes from glyphosate applications and take remedial action to prevent seed set. Therefore, it is important that the effect of weed management practices, particularly in glyphosate-resistant cotton, be examined for their influence on weed population dynamics and resistance selection. This will provide the ability to predict the likelihood and timeframe for resistance evolution under different weed management options and enable the possible altering of these strategies to reduce the risks of resistance.

This thesis takes the following steps in order to gain an understanding of how glyphosate resistance has influenced the cotton system thus far and to determine the potential for resistance evolution in glyphosate-resistant cotton under a range of management practices:

1. To investigate weed populations, weed management strategies and herbicide use patterns in Roundup Ready® and conventional cotton crops (Chapter 3).
2. To investigate the population dynamics of weeds in cotton systems, focusing on two key grass weeds, *Urochloa panicoides* Beauv. (liverseed grass) and *Echinochloa crus-galli* (L.) Beauv. (barnyardgrass), under a range of management practices (Chapter 4).
3. To further investigate the population dynamics of liverseed grass and barnyardgrass in terms of seed production and biomass (Chapter 5) and dose-mortality response to increasing rates of glyphosate (Chapter 6).
4. Develop a model from these experiments that will predict the likelihood of resistance evolution to glyphosate across a range of species and over a range of management strategies that will allow development of sustainable weed management practices utilizing Roundup Ready® Cotton (Chapter 7).

It is not the aim for this project to give a definitive answer for all weeds that are likely to be exposed to glyphosate but rather to estimate the level of resistance risk for weeds found in a Roundup Ready® cotton system utilizing two species as a starting point.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Herbicide resistant weeds have become a threat to conventional agricultural practices (Jasieniuk *et al*. 1996). In 2006, there were 183 weed species with populations resistant to a number of herbicide modes of action in 59 countries across the world (Heap 2006). Herbicide resistance is a consequence of the extensive use of herbicides for weed control in crop and fiber production systems (Preston and Powles 2002), particularly where one only a or few herbicides are used persistently to manage weeds (Preston and Reiger 2000). The reliance on herbicides has also resulted in shifts in the composition of weed species (Kudsk and Streibig 2003). Currently in Australia, populations of 32 weed species are resistant to herbicides from nine herbicide groups (Heap 2006). *Lolium rigidum* (annual ryegrass) is the most well known of these. Herbicide resistance first appeared in this species in 1980 (Heap and Knight 1982).

The introduction of herbicide-resistant crops (HRCs) has been hailed as the next revolutionary breakthrough in weed control (Baldwin 1999). The perceived benefits of HRCs have resulted in a rapid increase in the area of HRCs grown around the world despite the continuing debate over genetically modified organisms (Malik *et al*. 1989; Baldwin 1999). Widespread adoption of HRCs poses risks similar to those related to the introduction of a new herbicide that has the same mode of action as herbicides already in production (Wyse 1991). Herbicide-resistant crops carry with them a number of benefits and concerns ranging from improved flexibility of management of difficult-to-control weeds to possible adverse impacts on herbicide resistance evolution. These benefits/concerns will be discussed more fully in the section on herbicide tolerant crops (Section 2.3).

Glyphosate-resistant cotton (Roundup Ready®) is likely to have an effect on conventional weed management practices, the weed spectrum and the risks of glyphosate resistance evolving in Australian cotton farming systems. Glyphosate-resistant cotton can currently tolerate over-the-top applications of glyphosate (*N*-phosphonomethyl glycine) through the fourth true leaf stage (Monsanto 2000). Glyphosate is currently the most widely used and effective herbicide in cotton farming systems. It is used to control a wide range of broadleaf and grass weeds both within cotton and in fallow. There are no reported cases of glyphosate resistance in Australian cotton fields to date, however the introduction of glyphosate-resistant cotton could lead to a greater use of glyphosate on cotton fields which in turn may lead to herbicide resistance problems (Roberts 1999). The evolution of glyphosate-resistant *Lolium rigidum* (rigid ryegrass) in southern Australia is proof that resistance to all herbicides can evolve if they are used repeatedly (Roberts 1998*a*; Preston *et al*. 1999). The introduction of glyphosate resistance in crops may result in substitution of glyphosate for other weed management practices, thus increasing this risk (Roberts 1999).

The use of an integrated weed management (IWM) strategy is effective for the management of weeds as well as preventing the onset of resistance evolution. Integrated weed management involves the use of mechanical and cultural practices, such as tillage, chipping, crop rotation and crop competition (Nalewaja 1999), as well as rotating herbicide modes-of-action (Powles *et al*. 1997) to minimize the selection pressure placed on weeds that would arise from the continual use of a herbicide or herbicides with the same mode of action. Although the Australian cotton industry relies heavily on herbicides (Charles *et al*. 1995), conventional weed management strategies still include cultivation and hand-hoeing in addition to herbicides (Roberts 1998*b*). It is, therefore, important that the likely effects that glyphosate-tolerant cotton will have on integrated weed management practices be considered.

In order to gain sufficient understanding of the effects of these control practices, the population dynamics of the weed species needs to be examined (Jordan 1992). As failure to consider the underlying biology of the weed species is likely to result in incorrect predictions (Cousens 1985). This involves understanding factors such as germination characteristics, response to control measures, seed production, and seedbank dynamics.

Computer models are a valuable tool in the assessment of management strategies for their effects on weed populations. They can be used to evaluate strategies over the long term (Pannell *et al*. 2003) as opposed to trials in the field which take a long time to see results. Resistance models have been created for rigid ryegrass to assess management practices on resistance evolution (Preston and Roush 1998; Pannel *et al*. 2003). The accuracy of a model depends on the amount of data available to enter into the model (Preston and Roush 1998). Therefore, to develop a model for population dynamics in glyphosate-resistant cotton, it is important to use weeds where a substantial amount of data is either known or able to be gathered. *Echinochloa crus-galli* (barnyardgrass) and *Urochloa panicoides* (liverseed grass) are important weeds in northern Australian cropping systems (Felton *et al*. 1990; Felton et al. 1994), and they are very competitive with crops (Wiese and Vandiver 1970; Roberts 1999). These two species will therefore be useful in the development of a model to assess the effects of weed management practices on population dynamics and subsequently the probability of glyphosate resistance occurring in Australian cotton fields.

2.2 HERBICIDE RESISTANCE

2.2.1 Resistance defined

Herbicide resistance is defined as the evolved capacity of a previously susceptible weed population to survive and reproduce when a herbicide is used at its normal rate (Heap and LeBaron 2001). It is a decreased response of a population of plant species to a control agent as a result of its application (LeBaron and Gressel 1982). Resistance differs from tolerance, which is the natural and normal variability to tolerate application from pesticides that exists within a species (Heap and LeBaron 2001).

Herbicide resistance is thought to be caused by genetic mutations that result in a change to the way a plant responds to a herbicide (Friesen *et al*. 2000). There are different forms of resistance, depending on the mechanism involved. Target-site resistance is the result of a modification of the herbicide-binding site (usually an enzyme) which stops the herbicide from binding effectively (Heap and LeBaron 2001). Nontarget-site resistance is due to a mechanism(s) other than target-site modification. This can be via mechanisms, such as enhanced metabolism, reduced rate of herbicide translocation, sequestration and other mechanisms, that reduce the amount of herbicide reaching the target site (Heap and LeBaron 2001). Cross resistance is where a single resistance mechanism confers resistance to several herbicides; this can be target-site and nontarget-site resistance (Heap and LeBaron 2001). Multiple resistance occurs when two or more resistance mechanisms are present within individual plants of a population (Heap and LeBaron 2001). Polygenic resistance involves plant populations becoming more resistant with time by the accumulation of many genes, each giving a small effect (Preston and Roush 1998). Rigid ryegrass (*Lolium rigidum*) populations readily evolve multiple resistance. In southern Australia, populations of this species have resistance to herbicides from seven different herbicide mode of action groups (Heap 2006).

2.2.2 Evolution of herbicide resistance

The evolution of herbicide resistance in a weed population is is the result of an increase in frequency of a pre-existing resistance allele due to the selection pressure exerted by repeated herbicide applications (Betts *et al*. 1991). Hence, there are two factors that lead to the evolution of resistance: 1) heritable/genetic variation and 2) selection (Maxwell and Mortimer 1994).

2.2.2.1 Genetic Variation

The appropriate genetic variation on which selection can exert an effect will only be present in certain alleles. However, these alleles may not exist in all populations. Resistance traits can arise within a population by a major gene, or genes, which may be present at a low frequency (Maxwell and Mortimer 1994). Preston and Powles (2002) found the frequency of herbicide-resistant rigid ryegrass populations that had no previous herbicide exposure to be about 1 in 20 000. The evolution of resistance is more rapid in a population that contains resistant alleles before selection. When resistance alleles aren't present, resistance can only evolve if there is a new mutation (Maxwell and Mortimer 1994). Resistance can be associated with more than one trait, and this will effectively increase the rate of resistance evolution (Maxwell and Mortimer 1994). In populations that do not posses resistant alleles before selection, the onset of resistance by mutation is dependant on the size of the population and the mutation frequency (Maxwell and Mortimer 1994).

Resistant alleles may occur as a result of mutations causing changes at the herbicide site of action. A relatively minor change in a polypeptide sequence can result in a major change in herbicide affinity at the target site (Betts *et al*. 1994). This was the case with triazine resistance in *Amaranthus hybridus* L., where resistance is due to the change of a single amino acid in the polypeptide sequence containing the triazine binding site (Hirshberg and McIntosh 1983).

The seed bank plays an important role in the onset of resistance. Where there is a relatively rapid turnover of the seed bank, resistance can evolve more rapidly as the time between successive cohorts is shorter (Shaner 1995). The rate of turnover may be a characteristic of a species or aided by cultural practices. Changes in tillage practices may result in a more rapid turnover of the seed bank. Work by Cardina *et al*. (2002) showed that the number of seeds in the top 5 cm of soil is greater in no-till and chisel plough tillage programs as compared to moldboard ploughing. The introduction of new weed seeds into areas by the spreading of manure has been related to the spreading of triazine-resistant weeds from dairy producing areas into cropping areas in the United States (Shaner 1995).

2.2.2.2 Selection Pressure

Herbicide resistance has appeared in general where one or a few herbicides are used persistently to manage weeds (Preston and Rieger 2000). Selection pressure for herbicide resistance is affected by intensity of herbicide use, frequency of use and duration of the effect. Selection intensity is the relative mortality of target weeds and reduction in seed production. Selection duration is the time over which phytotoxicity is imposed by the herbicide. These factors interact to give seasonal variation in the selection pressure placed on weeds according to their phenology and growth (Maxwell and Mortimer 1994). Even if the frequency of genetic variation for resistance is very low, repeated herbicide applications will usually result in a rapid increase in the frequency of resistant individuals until they eventually dominate the population (Jasieniuk *et al*. 1996).

The question has been raised regarding the effect of herbicide rates on resistance. Preston and Roush (1998) state that the evolution of resistance is more dependant on the level of control achieved by a herbicide than the dose rate. In Australia, target-site based resistance mechanisms are just as readily selected with low rates as with high herbicide rates. Low rates allow the expression of weaker resistance mechanisms that won’t appear if high rates are used. However, these can be weak target-site mechanisms as easily as metabolism-based mechanisms (Preston and Roush 1998).

A major determinant in the selection of herbicide-resistant biotypes is the effective selection intensity that differentiates resistant individuals from susceptible ones in the face of selection (Maxwell and Mortimer 1994). This involves the term “fitness”, which is defined as the evolutionary advantage of a phenotype and is based on its survival and reproductive success. Fitness is expressed in relative terms, comparing alleles relative to the most successful one (Maxwell and Mortimer 1994). The fitness of the individuals containing resistant alleles and their ability to reproduce will have a large impact on the proportions of resistant and susceptible alleles in a population. In the case of triazine-resistant weeds, resistance results in impaired photosynthesis and, therefore, a "fitness cost" of resistance is that resistant genotypes have only 42% to 70% of the total biomass production compared with susceptible genotypes (Jasieniuk *et al*. 1996; Jordan 1999). The rate of increase or decrease of the resistant population would depend on the relative fitness disadvantage of the resistant genotype and its ability to acquire compensatory traits by crossing with the wild type. In the absence of a herbicide, the resistant genotype generally is less fit than the susceptible genotype. Conversely, when a herbicide is applied the resistant genotype survives and therefore will become a larger proportion of the population (Jordan 1999).

2.2.3 Glyphosate resistance

There are currently eight weed species with populations that are resistant to glyphosate. These are common ragweed (*Ambrosia artemisiifolia*), horseweed (*Conyza canadensis*) and palmer amaranth (*Amaranthus palmeri*) in the United States, buckhorn plantain (*Plantago lanceolata*) in South Africa, hairy fleabane (*Conyza bonariensis*) in South Africa and Spain, goosegrass (*Eleusine indica*) in Malaysia, Italian ryegrass (*Lolium multiflorum*) in Chile and the Unitied States, and rigid ryegrass (*Lolium rigidum*) in Australia, the US and South Africa (Heap 2006).

In 1997, a glyphosate resistant population of *Eleusine indica* was reported by a fruit grower in Malaysia, where glyphosate failed to provide adequate control in his four-year-old orchard (Lee and Ngim 2000). In this situation, herbicides were sprayed from six to eight times per year. This, plus the high fecundity of goosegrass, represents optimum conditions for the selection of a herbicide resistant allele in a weed population (Lee and Ngim 2000). One population of *Eleusine indica* from Malaysia was found to have a 5-enolpyruvylshilimate-3-phosate synthase (EPSPS) with reduced sensitivity to glyphosate. The resistant enzyme was five times less sensitive to glyphosate, while the plant was three times less sensitive. The mechanism conferring resistance to glyphosate in this population has been established as a target site modification with two different amino acid modifications identified in the gene for EPSPS (Tran *et al*. 1999).

In 2001, VanGessel reported resistance to glyphosate in populations of *Conyza canadensis* evolved glyphosate resistance within three years of growers using only glyphosate for weed control in glyphosate-resistant soybeans (*Glycine max* (L) Meer.) in the United States. Seedlings from a resistant population were found to have 8- to 13- fold glyphosate resistance. This was the first report of an annual broadleaf plant exhibiting glyphosate resistance due to repeated glyphosate applications (VanGessel 2001).

Glyphosate resistance in a population of rigid ryegrass evolved in an orchard in Australia following two to three applications of glyphosate for 15 years (Powles *et al*. 1998). The mechanism of resistance in rigid ryegrass is not due to reduced sensitivity of the EPSPS to glyphosate (Hawkes *et al*. 1999; Lorraine-Colwill *et al*. 1999). Research has been conducted on rigid ryegrass populations that found no difference in glyphosate absorption or metabolism with respect to susceptible populations. The enzyme DAHP (3-deoxy-D-arabino-heptulosonate-7-phosaphate) synthase, which is the first enzyme in the shikimate acid pathway, was also examined and found not to be different (Hawkes *et al*. 1999; Lorraine-Colwill *et al*. 1999). It is thought that resistance to rigid ryegrass is due to reduced movement of glyphosate to its site of action in the plastid (Hawkes *et al*. 1999; Lorraine-Colwill *et al*. 1999). Further research by Lorraine-Colwill et al. (2002) found that an alteration in the transport of glyphosate to the leaf tips of resistant plants as opposed to the roots of susceptible plants is correlated with glyphosate resistance. Wakelin *et al*. (2004) found that susceptible rigid ryegrass plants translocated twice as much herbicide to the stem meristematic portion of the plant as resistant plants, suggesting an association between glyphosate resistance in rigid ryegrass and the ability of glyphosate to accumulate in the shoot meristems. In Chile, resistance of *Lolium multiflorum* to glyphosate occurred in an orchard that had been treated three times per year for 8 to 10 years (Perez and Kogan 2003).

Glyphosate is used extensively in Australia in agricultural, industrial and domestic situations, with particular importance in dryland no-till areas. The use of glyphosate-resistant crops is likely to increase the incidence of glyphosate-resistant weed populations. It is also unlikely that a replacement for glyphosate will be available in the foreseeable future. Thus, it is imperative that strategies to delay the onset of glyphosate resistance in weeds are in place, as well as an increased understanding of the likely mechanisms conferring glyphosate resistance (Preston and Rieger 2000).

2.2.4 Current situation in Australia

The first case of herbicide resistance in Australia was reported in a population of *L. rigidum* resistant to diclofop-methyl in 1982 (Heap and Knight 1982). There are currently 32 weed species (Table 2.1) resistant to nine herbicide groups in Australia (Heap 2006).

The group B herbicides (sulfonylureas and imidazolinones) have the highest incidence of resistance, with 14 species having populations resistant to this group of herbicides. There is currently only one species with glyphosate (group M) resistance, rigid ryegrass; however it is estimated that this species has 54 resistant populations across New South Wales, South Australia, Victoria and Western Australia (Table 2.1).

NOTE: This table is included on page 14 of the print copy of the thesis held in the University of Adelaide Library.

Table 2.1. Herbicide-resistant weed species in Australia by mode of action group (Heap 2006; Preston and Reiger 2000)

NOTE: This table is included on page 15 of the print copy of the thesis held in the University of Adelaide Library.

Table 2.2. Incidence of confirmed glyphosate-resistant rigid ryegrass populations in Australia (Preston pers. comm.).

2.2.5 Using IWM to delay resistance

A major cause of the evolution of resistance, as stated earlier, is selection from the repeated use of one or a few herbicides with the same mode of action for weed control (Maxwell and Mortimer 1994; Preston and Rieger 2000). Therefore, a way to minimize this selection pressure is to reduce the reliance on herbicides by using IWM techniques. Herbicides will always play an important role in weed control systems as they are easy to use and cost effective (Powles *et al*. 1997). Rotating herbicide modes of action can be a viable strategy not only after resistance has occurred, but also to minimize the likelihood of resistance occurring (Powles *et al*. 1997). Shaner (1995) specified recommendations published by agrochemical companies, universities and extension for minimizing the risk of herbicide resistance:

1. Determine which weeds infest the crop fields or non-crop sites.
2. Use historical weed densities or weed thresholds, as appropriate, to determine the need for herbicide treatment to tailor the herbicide.

3. Use a diverse herbicide program that includes tank-mix or sequential treatments with herbicides that have different modes of action and that are effective on the same spectrum of weeds, especially those that are at highest risk of evolving resistance.
4. Use non-chemical weed control practices, such as tillage or mowing, in conjunction with herbicides whenever possible.
5. Rotate crops and use herbicides with different modes of action.
6. Discourage extended use of a single herbicide or herbicides with the same mode of action on the same field for control of the same weed.
7. Use certified crop seeds, and clean equipment when moving from one field to another to prevent spreading resistant weed seeds.

2.3 HERBICIDE-RESISTANT CROPS

2.3.1 Herbicide-resistant crop use

Currently there are a number of herbicide-resistant crops in use throughout the world and in Australia. This number continues to increase as suitable varieties become available. For example, in the United States in 1999 glyphosate and bromoxynil-resistant cotton made up over 50% of cotton area planted in some states (Baldwin 1999). Glyphosate-resistant cotton now makes up more than 80% of cotton planted in the United States (Powles and Preston 2006). There are herbicide-resistant varieties available of crops such as cotton, maize (*Zea mays* L.), soybean, wheat (*Triticum aestivum* L.) and canola (*Brassica napus* L.) (Harrison 1992; Baldwin 1999; Lyon *et al*. 2001). Herbicide-resistant crops are becoming an increasing part of Australian cropping systems, and an insight into HRCs on the market is as follows.

2.3.1.1 The Australian scene

Cotton has herbicide-resistant varieties genetically modified to tolerate glyphosate (Roberts 1999). Roundup Ready® cotton, developed by Monsanto, is now widely used in the cotton industry. Research has been conducted into 2,4-D-resistant cotton which could reduce the incidence of cotton damage due to 2,4-D drift (Charles *et al*. 1998). The release of these, along with glufosinate-resistant varieties and stacked combinations, will broaden post-emergent weed control options in cotton (Roberts 1999). It is unlikely in the near future that either 2,4-D-resistant cotton will be commercialised in Australia.

Canola is another crop that has widespread use of herbicide-resistant varieties. Triazine-resistant canola covered 60 to 70% of the canola producing area in 2002 (Roush and Preston 2002). Clearfield® canola, which is imidazolinone resistant, is also in use (Roush and Preston 2002). Other varieties resistant to glyphosate and glufosinate may also soon be available (Roush and Preston 2002).

BASF have developed a Clearfield® production system for wheat and maize in addition to canola. These crops are also tolerant to imidazolinone herbicides. Other prospects on the horizon include glyphosate-resistant wheat, which may soon be available in Canada (Blackshaw and Harker 2002), and more Roundup Ready® crops, such as soybeans and maize.

2.3.2 IWM impacts of herbicide-resistant crops

Weed management practices have the potential to change significantly as a result of the introduction of herbicide-resistant crops (Roush 2002). The impact of HRCs is likely to be the same as the introduction of a new herbicide that has the same mode of action as herbicides already in production (Wyse 1991). These impacts raise concerns with regard to weed resistance and weed species shifts (Wyse 1991; Forcella 1999; Derksen *et al*. 1999; Lyon *et al.* 2001).

2.3.2.1 Benefits of herbicide-resistant crops

Herbicide-resistant crops provide greater flexibility in weed management and new solutions to difficult weed management problems (Burnside 1992). They also provide new uses for existing herbicides, particularly for weed species that are naturally tolerant or increasingly resistant to selective herbicides (Roush 2002). This technology should complement existing weed management practices (Roberts 1998*b*). With increased flexibility of weed management available, growers have the ability to adopt multiple and integrated approaches in their programs (Burnside 1992). In North America, growers find HRCs make weed management easier, more effective and less expensive that previous weed control practices (Blackshaw and Harker 2002).

Herbicide-resistant crops used responsibly should encourage a transition towards using more environmentally benign herbicides, such as glyphosate (Burnside 1992). This provides greater flexibility in application with a greater spectrum of target weeds (Faircloth *et al*. 2001; Roberts 1999). The ability to substitute pre-plant residual herbicides that require incorporation with post-emergent herbicides will aid in soil conservation through a reduction in tillage. Therefore, soil exposure and erosion will be reduced (Burnside 1992; Roberts 1998*a*).

Herbicide-resistant crops provide another way the crop can be protected from herbicide damage (Hinchee *et al*. 1993; Charles *et al*. 1998). Chemical safeners and mechanical methods of protecting crops, such as shielded sprayers, are possible but can be expensive (Hinchee *et al*. 1993). Cotton is extremely susceptible to 2,4-D damage from drift and residues in spray equipment. The development of 2,4-D-resistant cotton would provide a solution to this (Charles *et al*. 1998).

2.3.2.2 Concerns of herbicide-resistant crops

A major concern with HTCs is the potential for increased use of herbicides (Burnside 1992; Roush 2002). Given that herbicides are already used extensively, this could pave the way for increased herbicide resistance (Roush 2002). In triazine-resistant canola, increased triazine resistance has been reported in annual ryegrass in WA due to atrazine use (Roush 2002). There is also concern, with rigid ryegrass already showing resistance to glyphosate in southern Australia, that the introduction of glyphosate-resistant varieties will encourage greater use of glyphosate and thus increase resistance (Roush 2002). Crop rotations where HRCs are present in more than one phase of the rotation may increase the selection pressure on weeds if the crops in rotation are tolerant to herbicides with similar modes of action. An example of this is glyphosate-resistant canola introduced into areas where glyphosate-resistant cotton is grown in summer. There is also concern with resistance to group B (ALS-inhibiting) herbicides evolving in imidazolinone-resistant canola (Roush 2002). Resistance to these herbicides can be selected with 4 to 5 uses (Preston and Roush 2002).

There is also concern that if HRCs are able to cross with weed species, this may facilitate the evolution of herbicide resistant weeds (Burnside 1992; Roush 2002). In Australia there are few crops that have wild relatives that they are able to interbreed with (Roush 2002). There have been concerns with canola, which potentially can hybridize with wild radish (*Raphanus raphanistrum*) and Buchan weed (*Hirschfeldia incana*). In studies conducted by Reiger *et al*. (2002), out of 50 million seeds screened, two plants were found that were hybrids on the basis of their herbicide resistance and chromosome numbers. These experiments suggest that the frequency of naturally occurring herbicide-resistance genes is likely to be greater than the frequency of cross-pollination.

The incidence of HRC volunteers is another issue that arises from the use of HRCs. There have been reports in Canada that hybridization of different herbicide resistance types from neighboring cultivars has created a problem (Roush 2002). Volunteer herbicide-resistant canola may even be considered to be a larger problem than the evolution of weed resistance (Derksen *et al*. 1999). In the Australian cotton industry, surveys have been conducted to monitor the incidence of Roundup Ready volunteers (Farrell and Roberts 2002; Perry 2002).

2.3.2.3 Weed populations and population dynamics

Shifts in weed species occur in response to changes in production practices or weed management systems (Lyon *et al*. 2001). Therefore, it is likely that the use of HRCs will cause shifts in weed populations. As changes in weed species have been a continuing feature of changes in cropping practices, the introduction of HRCs should be viewed no differently than any other change in weed management practices (Sommervaille 1996). Forcella (1999) states that HRCs will affect weed seed banks mainly through two interrelated factors. The first is application timing, and the second is reduced tillage.

Herbicides, such as glyphosate and glufosinate, which are used with HRCs have little or no residual activity. Therefore, if the herbicide is applied prior to full seedling emergence of weeds, then weed seed production and seed bank augmentation is likely. If the herbicide is applied too late, the seed bank can be enriched (Forcella 1999).

The environmental benefits of reduced tillage are well known. However, for weed management the benefits and costs are not consistent (Forcella 1999). Seedling emergence is often delayed when tillage intensity is reduced (Spandl *et al*. 1999). Systems that prolong the duration of weed emergence increase the importance of the herbicide application timing due to increased proportions of later-germinating weeds. These weeds may be harder to control with non-residual herbicides than earlier germinating weeds and thus may go to seed, replenishing seed banks (Forcella 1999). Changes are more likely to occur in reduced-tillage systems where there is a greater reliance on glyphosate, and therefore could compromise the sustainability of conservation tillage in dryland situations (Derksen *et al*. 1999).

In Canada, weed populations have changed through the adoption of canola varieties tolerant to glyphosate, glufosinate and imidazolinones, but long-term weed population dynamic changes have not occurred (Derksen *et al*. 1999). If the herbicides used on HRCs become the dominant selection pressure on weeds, there is also potential for adverse changes in weed composition due to increased frequency of canola within rotations in Canada (Derksen *et al*. 1999). A key to managing species shifts is to anticipate which species will survive a specific management system. Weeds could persist in HRC production systems because of tolerance to the herbicide or because of growth types or life cycles that allow them to avoid being treated (Madsen and Streibig 2002).

2.3.2.4 Changes in IWM practices

With the introduction of HRCs to particular agricultural systems, the practices adopted in those systems will affect the onset of resistance. Herbicide resistant crops provide greater options to rotate herbicides in crop and to use non-chemical weed control methods (Burnside 1992). Producers could adopt these methods or they could increase the use of a particular herbicide. In the latter case, Burnside (1992) suggests that it will be only a matter of time until herbicide-resistant weeds appear.

The impact HRCs have on weed dynamics and weed resistance will be determined by the intensity and frequency of selection pressures exerted on weed communities (Derksen *et al*. 1999). If different herbicide/cultivar systems are rotated within fields and problem weeds are managed in between, adverse changes in weed communities should not occur (Derksen *et al*. 1999). The rotation of herbicides, crops and other weed control methods, such as tillage, should prevent the increase of weeds that are resistant to a given weed management method (Burnside 1992).

Measures will also need to be taken to control any HRC volunteers. Some of these may include control of crop volunteers by herbicides with different modes of action, as is the case in control of Roundup Ready® volunteers with paraquat (Monsanto 2000). Practices, such as tillage (Monsanto 2000), isolation and hygiene (Roush 2002), are also effective methods of volunteer control.

Prediction of which weed species may present problems in herbicide-resistant systems would enable development of strategies to prevent weed invasion (Lyon *et al*. 2001). Weeds are combated best by a variety of weed control methods; however, these management systems require considerable planning and assessment of weed competitiveness in response to various control methods (Burnside 1991). The willingness of the producer to adopt multiple methods will determine the likelihood of weed species shift and resistance.

2.4 GLYPHOSATE-RESISTANT COTTON

2.4.1 Glyphosate mode of action

2.4.1.1 Translocation through the plant

Glyphosate is a systemic post-emergent herbicide (Baird *et al*. 1971) that primarily enters plants through the stems and leaves (Franz *et al*. 1997). Entry of glyphosate through plant roots is inhibited by tight adsorption of the herbicide to the soil (Malik *et al*. 1989). Glyphosate enters the leaf surface via diffusion through the cuticle; this is most likely via the hydrophilic pathway (Malik *et al*. 1989). Initial penetration of glyphosate is rapid to the amount of approximately 35% within the first few hours after application. This is then followed by a slow rate of penetration for the next few days (Malik *et al*. 1989).

Glyphosate is transported across cell membranes allowing entry into the symplast and movement through the vascular tissues (Malik *et al*. 1989). It is highly phloem mobile, and it is readily translocated with photosynthate in the same way as sucrose (Gougler and Geiger 1984; Duke 1988) to metabolically active regions such as meristems, fruits, nodules and storage tissues (Baird *et al*. 1971; Wyrill and Burnside 1976; Sandberg *et al*. 1980; Gougler and Geiger 1984; Duke 1988; Devine *et al*. 1993).

The growth stage at which glyphosate is applied affects the pattern of herbicide translocation. Pline *et al*. (2001) found that herbicide translocation in glyphosate-resistant cotton followed the pattern of photosynthate translocation in a developmental stage-dependant manner. Plants that received over-the-top treatments at the 4- and 8-leaf stages translocated glyphosate mainly to foliar sites, whereas plants receiving directed spray treatments at these stages translocated glyphosate to the root and stem tissue as well as foliar tissue. Plants receiving post-emergent applications at the 12-leaf stage translocated glyphosate primarily to the stem, fruiting branches, and leaves. Therefore, during reproductive growth, glyphosate, like photosynthate, did not accumulate at tissues that were sinks at vegetative stages (Pline *et al*. 2001).

2.4.1.2 Action at the target site

Upon translocation to metabolically active regions of shoot and root tips (Baird *et al*. 1971), glyphosate inhibits the biosynthesis of aromatic amino acids (Kishore *et al*. 1991). Plants synthesize all of their amino acids. The shikimate pathway is the route of biosynthesis for phenyalanine, tyrosine, and tryptophan. There are also many aromatic secondary plant products, such as lignins, alkaloids, flavonoids and benzoic acids, that are important in plant growth and development and in interactions with other organisms that are also products of this pathway (Devine *et al*. 1993).

The target enzymes for many herbicides are compartmentalized within the chloroplast (Daniell *et al*. 1998). Glyphosate is the only herbicide that attacks EPSPS, an enzyme of the shikimate pathway. This pathway is present in plants, bacteria and fungi (Padgette *et al*. 1995). All the enzymes of the shikimate pathway have been found in the plastid (chloroplast); however, cytosolic forms of some enzymes apparently exist (Della-Cioppa *et al*. 1986; Devine *et al*. 1993). The plastid enzymes are highly regulated, whereas those of the cytosol are under less control (Devine *et al*. 1993).

5-enolpyruvylshikimate-3-phosphate is common to the synthesis of the aromatic amino acids, tyrosine, phenylalanine, and tryptophan which are essential for critical processes including cell wall formation, defense against pathogens and insects, production of hormones, and production of compounds required in energy production (Padgette *et al*. 1995). It is estimated that nearly 40 to 60% of the carbon fixed by plants moves through the shikimate acid pathway (Kishore *et al*. 1991).

5-enolpyruvylshikimate-3-phosphate catalyzes the reversible reaction of S3P (shikimate-3-phosphate) and PEP (phosphoenolpryuvate) to produce EPSP (5-enolpyruvylshikimate-3-phosphate) and Pi (inorganic phosphate) (Padgette *et al*. 1995). Glyphosate binds to EPSPS as the EPSPS-S3P-glyphosate complex, and this "dead-end" complex prevents the EPSPS reaction from proceeding to form products (Padgette *et al*. 1995). This leads to deregulation of the shikimate pathway, as well as accumulation of very high levels of shikimate acid (Devine *et al*. 1993). The reasons for plant death are uncertain, but may include disruptions of carbon partitioning (Geiger *et al*. 1986), build up of shikimate or loss of crucial secondary products (Devine *et al*. 1993).

2.4.2 Glyphosate resistance

2.4.2.1 Mechanisms of resistance

Research into the production of crops that are resistant to glyphosate began in the early 1980s (Padgette *et al*. 1995). The folowing three basic mechanisms that were tried to create resistance: 1. overproduction of EPSPS (Shah *et al*. 1986); 2. introduction of an EPSPS with decreased affinity for glyphosate (Barry *et al*. 1992; Comai *et al*. 1983; Hinchee *et al*. 1993); and 3. introduction of a glyphosate degradation gene (Barry *et al*. 1992). Over expression of a glyphosate-sensitive EPSPS in petunia (*petunia hybrida*) plants conferred glyphosate tolerance; however, the level of tolerance was not sufficient to withstand commercial glyphosate application rates (Padgette *et al*. 1995). In order to obtain higher levels of tolerance to glyphosate, different mechanisms needed to be developed. The introduction of glyphosate-insensitive EPSPS into crops by genetic modification techniques proved to be successful in imparting glyphosate tolerance. Glyphosate treatment of plants expressing glyphosate-tolerant EPSPSs did not cause damage due to the continued action of the glyphosate-tolerant enzyme to meet the need for aromatic amino acids (Padgette *et al*. 1995).

2.4.2.2 Development of Roundup Ready® cotton

There has been research on various glyphosate-tolerant EPSPSs. The isolation of glyphosate-tolerant mutants of *Salmonella typhimurium* was conducted by Comai *et al*. (1983). A variant EPSPS isolated from *E. coli* for growth in the presence of glyphosate (Kishore *et al*. 1986) was cloned and shown to contain an amino acid substitution G96A. This strain showed an 8000-fold decrease in glyphosate sensitivity compared with the wild-type *E. coli*.

Schultz *et al*. (1985) reported that EPSPSs from a number of bacteria exhibited tolerance to glyphosate. As a result, bacterial cultures from a wide range of sources were collected and analyzed for the ability of their EPSPS to tolerate to glyphosate. The EPSPS with the highest tolerance found was CP4 EPSPS. Based on kinetic parameters and suitability for use in conferring glyphosate tolerance to crops, the gene for CP4 EPSPS was cloned from *Agrobacterium* sp. strain CP4. The CP4 EPSPS coding sequence was then fused to CTP (chloroplast transit peptide) coding sequences to target the protein to the plastids of plants (Padgette *et al*. 1995).

Glyphosate-resistant cotton lines were developed by transfer of the gene encoding the EPSPS isolate from *Agrobacterium* sp. CP4 (CP4-EPSPS) to cultivar Coker 312 (Nida *et al*. 1996). These lines were then screened with greenhouse spray tests and field evaluations to find commercially acceptable lines (Nida *et al*. 1996). The acceptable variety was then crossed with elite commercial varieties through a process of backcrossing with the elite variety for several generations (Monsanto 2000). Two to five backcrosses were used to develop the commercially acceptable glyphosate-resistant varieties (Monsanto 2000).

2.4.3 Glyphosate-resistant cotton and the cotton industry

2.4.3.1 Effect on IWM practices

Glyphosate-resistant cotton provides increased flexibility in weed management options, allowing broad-spectrum post-emergent herbicide use and the adoption of conservation tillage practices (Jones and Snipes 1999; Faircloth *et al*. 2001). Preston and Reiger (2000) state that all HRCs will impact on the management of existing herbicide-resistant weed populations and on the potential for further resistance evolution in weeds. There are currently no reported cases of weed resistance in Australian cotton fields (Roberts 1998*a*). However, the question remains: will glyphosate tolerance lead to herbicide resistance problems in the Australian cotton industry? (Roberts 1999). A major factor in the evolution of herbicide resistance is the repeated use of herbicides with the same mode of action (Betts *et al*. 1991; Maxwell and Mortimer 1994; Shaner 1995; Preston and Reiger 2000), therefore the increased use of glyphosate as a substitution or replacement for other herbicides, mechanical or cultural methods will put the system at risk (Roberts 1999).

Welch *et al*. (1997) conducted trials in cotton comparing pre-plant incorporated (trifluralin) and pre-emergent (fluometuron) herbicides with glyphosate-only treatments and found that glyphosate-only treatments delivered higher yields than traditional herbicide programs. In other trials, glyphosate-only herbicide programs resulted in equal yields and higher returns in glyphosate-resistant cotton (Mills and Voth 1997; Webster *et al*. 1999; Wilcut and Hinton 1997). Damage to cotton seedlings from rainfall washing pre-emergent herbicides into the seed zone is also a problem (Charles *et al*. 1995). Factors such as these may result in the substitution of glyphosate for pre-emergent residual herbicides (Roberts 1999), and that will increase the threat of resistance to glyphosate. However, glyphosate-only treatment will not consistently produce higher yields and greater profits. Isgett *et al*. (1997) found that glyphosate used in conjunction with residual herbicides provided excellent weed control and high yields.

Tillage and hand-hoeing will continue to be valuable tools in glyphosate-resistant cotton. Mechanical weed control methods provide non-herbicide control options to which no resistance can develop (Roberts 1998*a*). However, one of the major selling points of HTCs is the capacity for reduced tillage (Forcella 1999) and chipping is generally expensive. A better result will be obtained if mechanical methods can be reduced, but not omitted, and used at appropriate times for control of survivors from herbicide programs (Roberts 1998*a*).

Glyphosate resistance in crops provides a useful tool for managing herbicide-resistant weeds. With proper integration of glyphosate-tolerant crops into a total weed management program, the selection of herbicide-resistant weeds could be minimized. However, if glyphosate were to frequently replace other herbicides, this would create problems (Shaner 2000). Preston and Reiger (2000) stated that the simplest strategy to delay the evolution of resistance in glyphosate-resistant crops would be to use a herbicide other than glyphosate pre-plant when glyphosate-resistant crops are grown or an alternative to glyphosate in years when the glyphosate-resistant crop is not grown. Irrigated cotton growers, however, do have the option to alternate weed control methods in crop. Monsanto has set guidelines stating that in, an alternative method of weed control must be used Roundup Ready® cotton to prevent seed set from weeds that have had exposure to Roundup Ready® herbicide. Methods included for alternative control are re-hilling, bed formation, hand-weeding, spot-spraying, inter-row cultivation and use of herbicides with a different mode of action (Monsanto 2000).

2.4.3.2 Limitations and New Technology

Glyphosate use in Roundup Ready® cotton is restricted to over-the-top applications no later than the four-leaf development stage (Jones and Snipes 1999). From this growth stage, applications must be made only by directed or shielded spray so there is no foliar contact (Monsanto 2000). This switch to directed application has several drawbacks. Weeds left within the plant line that can be shielded by the cotton plant or don't receive enough herbicide for control need to be controlled by other methods (Roberts 1999). This limitation is set due to glyphosate affecting pollen development, resulting in poor pollen growth on the stigma, as well as production of pollen with reduced viability (Pline *et al*. 2002). This is the likely explanation for increased boll abortion and pollination problems in glyphosate-resistant cotton treated topically after the four-leaf stage (Pline *et al*. 2002). The cotton plant appears to compensate for the loss of early season fruit; however, harvesting and weather related yield losses are likely to happen as a result of the delay in maturity (Jones and Snipes 1999).

Enhanced glyphosate-resistant technology has since been developed with better expression of the glyphosate resistance trait in reproductive parts of the cotton plant (May *et al*. 2004). Therefore, the application of glyphosate over-the-top on this glyphosate-resistant cotton can occur up to the 16 node stage. This new technology is likely to be released after 2006 with the trade name Roundup Ready Flex® (May *et al*. 2004). This will place increased importance on the continued adoption of IWM strategies for weed management.

2.5 INTEGRATED WEED MANAGEMENT

2.5.1 What is Integrated Weed Management

Integrated Weed Management (IWM) is the planned and combined use of physical, chemical and biological methods to control weeds (Powles and Matthews 1992). Integrated weed management is an important part of Integrated Pest Management (IPM), the development of which is in response to problems that have arisen in agriculture resulting in part from the overuse of pesticides (Wilson 2000). Weeds require specific attention for a number of reasons. Firstly, weeds differ from insects in mobility, population dynamics, effect on crop yield and the types of unintended side effects of their control (Goddard *et al*. 1995). Secondly, there is a different range of options for their control, such as greater use of non-chemical means in agricultural systems; and thirdly, due to their economic significance, specific attention applied to weeds is justified (Goddard *et al*. 1995).

An effective IWM system requires the integration of crop and weed biology with management tactics. This approach, therefore, increases the demand for managerial skills and can require more time than conventional weed management (Vengessel *et al*. 1996). The spectacular success of herbicides has led to the reduction of IWM practices on species easily controlled by herbicides. The appearance of multiple herbicide-resistant biotypes will force the adoption of IWM (Powles and Matthews 1992).

2.5.2 Reasons for adopting IWM

There are a number of reasons for adopting IWM systems. These include reducing the reliance on chemical or herbicide use, manageing herbicide resistance in the hope to delay its onset and, to manageing or controlling weed species shift towards herbicide-resistant weeds.

2.5.2.1 Reducing herbicide use

The increase in environmental and economic demands placed on farming systems provides strong incentives for reducing herbicide rates. Whilst growers may not place high importance on some of the perceived environmental effects of herbicide use, they are generally interested in measures that may reduce costs and maintain yields (Baldwin and Oliver 1985; DeFelice *et al*. 1989).

The objective of weed control is not to eradicate weeds, rather to reduce the density to levels that do not cause an economic impact (Burnside 1991). Weed management is best approached with a variety of weed control practices; however, this requires planning and assessment of weed responses to various control methods (Burnside 1991). The adoption of multiple weed management tactics in IWM systems and reduction of herbicide rates should enable producers to maintain high levels of weed control and reduce costs and residues associated with herbicides (VenGessel *et al*. 1996). The result would be increased profits and reduced amounts of herbicides in the environment (Norris *et al*. 2000).

2.5.2.2 Delaying herbicide resistance

Herbicide resistance evolves due to the continual use of a herbicide on a weed population, increasing the selection pressure for resistance to that herbicide. Through continual use of a herbicide, the percentage of individuals that are naturally resistant to that herbicide increases to a point where resistant individuals dominate the population (Roberts 2001).

An integrated approach to the management of herbicide resistance that uses various tools to decrease the selection of resistant weeds is required. Minimizing the continuous use of herbicides with the same mode of action is one of the key steps in resistance management (Shaner *et al*. 1999). The use of mechanical and cultural practices, such as tillage, hand-hoeing, crop rotations, delayed planting, and crop competition, to replace herbicides provide an opportunity to reduce the selection pressure that causes weeds to become resistant to herbicides (Nalewaja 1999). The reverse is also true; any weeds that may be tolerant to these mechanical or cultural methods can have the selection pressure reduced by herbicides. Therefore, rotation of these management practices should delay the resistance of weeds to both systems (Nalewaja 1999). For example, Matthews *et al*. (1996) found that in the case of herbicide-resistant rigid ryegrass in southern Australia, integrating the low risk herbicide management options of pre-emergent trifluralin application and crop-topping with competitive crop species, delayed sowing and seed catching showed good prospects for the long-term management of rigid ryegrass populations.

2.5.2.3 Weed species shift

Species shifts occur when the repeated use of a small number (predominately one) of weed control methods select populations that are not controlled well by these methods (Roberts 2001). The most common example of this is the continual reliance on one herbicide to control a mixed population of weeds (Roberts 2001).

Management practices such as tillage, rotations, and herbicides act as filters that determine the composition and abundance of weed species in fields. These management practices can filter out specific plant characteristics and have an effect of the direction of species change (Cardina *et al*. 2002). Further, management practices interact to determine the composition and abundance of the seed bank. The seed bank reflects past and current management while providing a picture of future weed species (Cardina *et al*. 2002). The challenge then for managers is to anticipate how changes in tillage, herbicide use, or rotation are likely to select for certain weeds or types of weeds in order to avoid troublesome and potentially resistant species (Cardina *et al*. 2002).

2.5.3 Integrated Weed Management practices

Integrated weed management involves practices such as tillage, crop rotation, crop competition, biological control, herbicides and, more recently, herbicide-resistant crops. These practices possess their own benefits and problems, as well as having different effects on weed species abundance and composition. However, they are often interrelated, and combinations of practices can influence weed species selection.

2.5.3.1 Tillage

Tillage has been the most widely used method of weed control since the dawn of agriculture. However, issues such as soil erosion, soil structure decline and water conservation have become major concerns in recent years and have created an interest in conservation tillage (Toler *et al*. 2002). Tillage is still a valuable tool in weed management in terms of seedbank dynamics (Pareja *et al*. 1985; Webster and Coble 1996) and reducing selection pressure of herbicides (Boerboom 1999; Nalewaja 1999).

The effectiveness of herbicides can be influenced by tillage through alterations in the distribution of weed seeds in the soil profile. Hartzler and Roth (1992) found that herbicides were generally more effective for weed control in conventional tillage than in no-tillage systems. Systems with intensive tillage operations distribute seed through the plow depth, whereas in no-till systems the seeds remain near the soil surface (Pareja *et al*. 1985). This was also the case in trials conducted by Cardina *et al*. (2002) in Ohio where it was found that in no-till plots the total number of seeds in the soil profile were higher than in chisel ploughed and moldboard ploughed plots. There were also four times as many seeds in the top 5 cm than at 5 to 10 cm and six times as many as at 15 to 20 cm (Figure 2.1). They also found that the distribution of seeds in the moldboard and to a lesser extent the chisel ploughed plots were relatively even.

NOTE: This figure is included on page 32 of the print copy of the thesis held in the University of Adelaide Library.

Figure 2.1. Number of germinable seeds at four soil depths in no-tillage (NT), chisel plow (CP), and moldboard plow (MP) (Cardina *et al.* 2002)

The type of tillage system, whether conventional or reduced, may also lessen the weed selection pressure compared to no-till systems due to the reduced reliance on herbicides and, potentially, lower weed densities. Mechanical weed control is a practical method to reduce the frequency and dose rate of herbicide use (Boerboom 1999) because systems that use fewer tillage operations are in danger of allowing weeds that can't survive frequent soil disturbance to invade and become problem weeds (Webster and Coble 1996). When assessing ways to reduce weed selection pressure, the potential benefits of conventional, minimum and no-till systems need to be weighed against the soil and water conserving benefits of reduced tillage systems (Boerboom 1999). With no-till systems, other resistance-delaying practices will need to be used to compensate for the increased risks inherent in no-till systems.

2.5.3.2 Crop rotation

Crop rotation aids disruption of weed life cycles due to different weed management practices applied and the different growth characteristics of each crop (Nalewaja 1999). This practice strategically applied can also be important in the control of problem weeds (Roberts 1998*a*). Where crops are grown continuously in monoculture, herbicides and other weed management practices are usually repeated. This has resulted in the selection of herbicide resistance (Boerboom 1999). A variety of herbicides with different modes of action are registered for some crops whereas other crops may have herbicides with only one mode of action registered for use (Nalewaja 1999). Therefore, the rotation of crops and potential for rotation of herbicide modes of action could reduce the selection pressure in the off year for a specific herbicide. This can be used to reduce herbicide use and therefore selection pressure over a number of years (Boerboom 1999).

Crop rotation and tillage type are some of the weed management practices that affect the size and composition of the weed seed bank. Crop-fallow practices also have an effect on weed communities, as was found in a trial conducted by Derksen *et al*. (1993). They studied the impact of tillage intensity and crop-fallow rotations on weed communities and reported that weed community differences were influenced to a greater extent by the inclusion or exclusion of fallow within rotations than by tillage systems. Weed control in crop or fallow situations impacts heavily on subsequent crops, as weeds that survive and produce seeds in one crop contribute to the seed bank, which can create problems in subsequent crops (Cardina *et al*. 2002).

2.5.3.3 Crop competition

Crop competition with weeds can be an effective method of weed management. Practices such as planting in narrower rows or at higher densities can effectively increase competition with weeds and reduce herbicide dose rate and frequency required for control (Boerboom 1999). Evenly planted populations also aid in weed control. Crop canopies can also restrict light to weeds in the inter-row spaces (Corbin and Pratley 1988). Another benefit of earlier canopy development is the shading effect, reducing evaporation that makes more moisture available to the crop (Corbin and Prately 1988). Askew and Wilcut (2002) found that the presence of cotton reduced the biomass of ladysthumb (*Polygonum persicaria* L.) by four times.

Reducing herbicide use, and therefore selection pressure, could be achieved if planting was delayed until after the major flushes of emerging weeds. However, in many crops, the risk of reducing yield potential may make this practice uneconomical (Boerboom 1999). In the United States, the practice of delayed planting of spring wheat was used to help manage wild oats (*Avena fatua* L.) before the introduction of herbicides (Nalewaja 1999). Although there were substantial increases in yield from the introduction of herbicides, when considering resistance and herbicide selection, the reduction in yield may be offset by the ability to reduce the reliance on herbicides (Nalewaja 1999).

Work by Lemerle *et al*. (2001) looked at the competitive advantage of wheat cultivars against rigid ryegrass. They found that varieties which showed a competitive yield advantage also suppressed rigid ryegrass. These studies indicated that selection for competitiveness would be beneficial, and that manipulating crop agronomic factors such as seeding rate may be a practical alternative.

2.5.3.4 Biological control

Biological control is still a relatively minor practice in current cropping systems, but it may be supplemental to other current practices in an overall management approach and has potential to become a major tool for weed management in integrated systems (Aldrich 1984). Insects and fungi have traditionally been used in biological control of noxious weeds (Kremer and Kennedy 1994). More recently, mycoherbicides, such as deleterious rhizobacteria, have had successfully controled specific weeds in some areas. The focus of research into these agents is on efficacy of those currently in use, and screening of new agents (Kremer and Kennedy 1994).

Biological control of weeds in Australia has had limited success since the introduction of cactoblastis (*Cactoblastis cactorum*) to control prickly pear (Storrie 2001). Australia has stringent restrictions on the import and release of control agents in the country. Biocontrol also targets single species, which can be a limitation (Storrie 2001). Unlike other weed control methods, the success of biocontrol agents is influenced greatly by the external environment (Van Tuat *et al*. 1999) or soil factors (Kremer and Kennedy 1994). In general, biocontrol is more suited to pasture systems due to lower levels of disturbance than cropping systems.

2.5.3.5 Herbicides

Chemical weed control provides an effective and feasible method of crop protection (Banyer *et al*. 1988). Herbicides have had a major positive impact on world agricultural production since the introduction of 2,4-D and MCPA in 1947 (Heap and LeBaron 2001). A significant proportion of Australian cropping systems have minimal crop rotation. Monocultures that result in pest populations with similar ecological requirements as the crop being grown are common. These pests often cannot be controlled without the use of pesticides (Banyer *et al*. 1988). In a trial comparing weed control methods in maize, Banyer *et al*. (1988) reported a 96:1 energy output to input ratio with herbicides compared to a 57:1 ratio for tillage and hand-hoeing control methods. Therefore, herbicides provide a very effective method of weed control and consequently yield increase for relatively less input than other weed control methods.

The effectiveness of weed management is greatly improved by the addition of herbicides. In a trial where herbicides were used continuously over a 6-year period, the weed seed bank was reduced by 96% (Vangessel *et al*. 1996). In a survey conducted by Hatzler *et al*. (1993) in the United States, cultivation alone resulted in denser weed populations than herbicide treatments at 41% of sites surveyed. Yield and economic returns are generally higher when herbicides are combined with mechanical treatments compared to mechanical treatments alone (Mulder and Doll 1992). Herbicides also provide flexibility in weed management (Mulder and Doll 1992; Hinchee *et al*. 1993).

The benefits of herbicides to weed management are obvious; hence, they are used extensively across the world in crop and fiber productions (Preston and Powles 2002). In Australia, the trend towards reduced cultivation, stubble retention, and permanent beds relies heavily on the effectiveness of herbicide applications (Roberts 1998*a*). The result of extensive herbicide use is the threat of herbicide resistance, which is now found in a number of weed species (Preston and Powles 2002). With the introduction of herbicide-resistant crops it is essential that the benefits of herbicide use are not lost due to herbicide resistance in weeds.

2.5.3.6 Herbicide-resistant crops

Herbicide-resistant crops promise to represent the next revolutionary breakthrough in weed control (Baldwin 1999). The interest in the development of HRCs has been influenced by a reduction in the rate of introduction of new herbicide compounds, the increased costs of developing new herbicides and advances in biotechnology and gene insertion (Harrison 1992; Roberts 1998*b*). The benefits/concerns of HRCs have previously been discussed. In relation to this project, the impact of HRCs on herbicide resistance, weed population dynamics and impacts to integrated weed management practices are important.

2.6 THE AUSTRALIAN COTTON INDUSTRY

In the 2004-05, season approximately 320 900 ha of cotton was grown in Australia, with 162 800 ha grown in New South Wales and 158 100 ha grown in Queensland (ABARE 2006). The major areas of cotton production include the Macquarie, Namoi, and Gwydir valleys in NSW, and the MacIntyre, Darling Downs, Emerald and Theodore in Queensland (Cotton Australia 2002).

Lint yield in 2001-02 was 358 000 tons in NSW and 297 400 tons in Queensland (ABARE 2006). Australia is the third largest exporter of raw cotton in the world, and Australian cotton accounts for around 13% of world trade. Up to 95% of Australian raw cotton is exported (CRDC 2002). In 2005, Australia exported approximately 409.6 kilo tons of lint to the value of $770 million (ABARE 2006).

2.6.1 Weed management in cotton

2.6.1.1 Current practices and herbicide use

Since 1997, herbicides have been classified according to their mode of action, with labels displaying a letter denoting the mode of action the herbicide. This was a step taken by the Herbicide Resistance Action Committee (HRAC) as part of a strategy to manage herbicide-resistant weeds. This enables farmers to plan their herbicide programs to avoid continually using herbicides with the same mode of action (MOA). In 1998, the Kondinin Group conducted a survey to determine farmer attitudes toward the MOA labeling. They found that most farmers in Australia are aware of the label and those who have resistance problems are using it in planning their weed management programs (Shaner *et al*. 1999). Table 2.3 contains a list of chemicals used in the cotton industry and their classification according to their mode of action.

NOTE: This table is included on page 38 of the print copy of the thesis held in the University of Adelaide Library.

Table 2.3. Herbicides classified according to mode of action that are common to cotton rotations (Roberts 1998*b*)

The cotton industry in Australia relies heavily on intensive management and pesticide use. Herbicides make up a large proportion of this (Charles *et al.* 1995). In general, high levels of weed control are achieved as most producers strive for weed-free fields (Charles *et al.* 1995) and have developed a “zero tolerance” attitude towards weeds (Roberts 1998*b*). This level of weed control employs intensive residual herbicide use, inter-row cultivation, hand chipping and crop rotations. The general result is low densities of weeds escaping with limited post-emergent herbicide controls to prevent seed set of these escaping weeds (Roberts 1998*b*).

In most cases, adequate control of grass weeds has been achieved by pre-emergent grass herbicides such as trifluralin and pendimethalin (group D herbicides), and metolachlor (group K herbicide) (Charles *et al.* 1995). The use of pre-plant herbicides such as diuron and fluometuron (both group C) help to improve weed control; however, the movement of these herbicides into the cotton seed zone, particularly following a rainfall event during emergence, results in damage to or death of cotton seedlings (Charles *et al.* 1995). Most cotton fields receive at least one application of herbicides from groups C and D (Roberts 1998b)

Broadleaf weeds have posed a greater problem, with few of these weeds being reliably controlled with available post-emergent herbicides (Charles *et al*. 1995). The recent introduction of pyrithiobac-sodium (Staple®) and trifloxysulfuron-sodium (Envoke®), both group B ALS-inhibiting herbicides, has aided in post-emergent control of broadleaf weeds. However, these herbicides are of high risk for selecting herbicide-resistant weeds due to their mode of action (Roberts 1998*b*).

Glyphosate is a non-selective post-emergent herbicide that is widely used in agriculture and in the cotton industry (Roberts 1998*a*). It is a group M chemical and its mode of action puts it in the low risk category for resistance. Glyphosate is one of the least likely herbicides to which resistance will evolve, however, the evolution of glyphosate resistant rigid ryegrass (Preston *et al*. 1999) has demonstrated that resistance can evolve to any chemical if it is used repeatedly (Roberts 1998^{a}).

Increased concern about soil and water conservation is leading to practices such as permanent beds, reduced cultivation and stubble retention (Roberts 1998*b*). This increases reliance on herbicides for weed control and increases selection pressure for resistance. Herbicide resistance has not yet been found in cotton fields; however, there is a significant risk of this occurring if the above IWM practices are reduced (Roberts 1998*b*).

2.6.1.2 Integrated Weed Management in cotton

The current weed management strategies employed in cotton farming systems may be described as an integrated weed management system as it employs a number of different weed management tactics although it is not ideal. Cultivation and chipping are used alongside herbicides for weed control (Roberts 1998*b*). Although cultivation is a beneficial tool with regard to weed control (Snipes *et al*. 1992), its use to control weeds in fallow and crop is not seen as a long-term sustainable solution (Roberts 1998*b*). Toler *et al*. (2002) evaluated weed and cotton response to various weed management systems with reduced-tillage in cotton. They found that optimum cotton production with reduced tillage could be achieved with the use of pre-emergent and early post-emergent herbicides. They also found that neither cultivation or herbicide treatments alone gave effective weed control (Toler *et al*. 2002).

There has been a decrease in hand-hoeing and cultivation in cotton crops since 1989 with an increase in the use of broadleaf, fallow and irrigation channel herbicides (Charles *et al*. 1995). Hand-hoeing, although expensive, is a valuable tool in the control of weeds in the plant line where they escape applications of residual and post-emergent herbicides. This mechanical control method has been a valuable tool in the prevention of herbicide resistance (Roberts 1998*b*). A possible solution to the expense of chipping is to use it late in the season to control escapes (Roberts 1998*a*) and prevent seed bank augmentation.

The role of crop rotation is another important factor in weed management as crops with different characteristics provide the opportunity to use different herbicides with different modes of action and to include other weed management options. There is evidence that nutgrass (*Cyperus* spp.) control can be aided by rotation with competitive winter cereals (Roberts 1998). Barrentine *et al*. (1992) recommended using crop rotations and alternating herbicides with different modes of action to control herbicide-resistant johnsongrass (*Sorghum halepense*) in the United States.

Ultra-narrow-row (UNR) cotton has shown potential to reduce input costs while producing acceptable yields (McCloskey *et al*. 2000). Cotton is typically grown in rows approximately 1m apart. Ultra-narrow-row cotton involves decreasing the row spacing to around 18 to 25 cm due to the potential for and increase in yield (Culpepper and York 1998). However, the inability to control weeds with post-emergent herbicides made UNR cotton unfeasible to produce (McCloskey *et al*. 2000). With the introduction of herbicide-resistant cotton varieties, researchers have re-evaluated weed control in UNR systems and found that adequate weed control can be obtained (McCloskey *et al*. 2000; Fowler *et al*. 1999).

Good IWM practices can be incorporated into the cotton system with the wide range of weed control options available (Roberts 1998). Certainly, herbicide-resistant cotton has increased these options (Burnside 1992). However, HTCs still need to become a part of a complete weed management system including pre-emergent residual and post-emergent herbicides, inter-row cultivation, chipping and appropriate rotation of both crops and herbicides (Roberts 1998). Good field records are also important in determining problem weeds and appropriate weed control methods (Roberts 1998).

<u>2.6.2 Weed management economics in cotton</u>

Weed management has historically been directed to prevent yield loss through competition. As a result, weed control decision making frameworks, such as economic thresholds, have been developed (Jones and Medd 2000). The economic threshold is the weed density where the cost of control using a fixed dose rate is equal to the benefits of control in terms of reducing economic injury (Jones and Medd 2000). Initially, the static approach was used where weed thresholds only considered current year effects of herbicide application. Long-term or dynamic approaches take into consideration weed population dynamics, factors such as seed bank replenishment over a number of years, and therefore are a better approach to economic thresholds (Jones and Medd 2000). This is a useful tool in management decisions; however, weeds that produce large volumes of seed, such as barnyard grass, present a risk if economic thresholds are used. For weeds such as these, small numbers of plants not controlled can replenish the seed bank (Bosnic and Swanton 1997).

In general, cotton provides the highest gross margins for broadacre crops, especially in irrigated circumstances. The gross margins of irrigated cotton for northern NSW in 2002-03 were approximately \$1500 ha^{-1}, compared to \$370 ha^{-1} for dryland cotton (Scott 2002). Typical costs for weed management in cotton are shown in Table 2.4.

NOTE: This table is included on page 42 of the print copy of the thesis held in the University of Adelaide Library.

Table 2.4. Cost of weed management operations in cotton (NSW Ag 2002)

This comparison demonstrates that even when the cost of the license fee for Roundup Ready® cotton is considered, weed management strategies that use fewer tactics such as chipping and pre- and post-emergent herbicides as a result of glyphosate applications in crop are cost effective. However, when making management decisions, growers need to consider the full impact of weed management options on the rotation. For example, Wiese *et al*. (1992) conducted a trial in the United States looking at weed management options and the effect on lint yield and profit. In irrigated cotton, they found that profits ranged from \$613 ha^{-1} with glyphosate alone and \$707 ha^{-1} with cultivation alone to \$751 when glyphosate was used after wheat, which was followed by cultivation and incorporation of trifluralin before planting cotton (Wiese *et al*. 1992). Furthermore, repeated use of a single herbicide mode of action will increase the development of herbicide resistance. Farmers may have to forego some income in order to prevent this. This is where the ability to predict resistance becomes important (Orson 1999).

2.6.3 Roundup Ready® cotton

Monsanto developed glyphosate-resistant (Roundup Ready®) cotton which was approved for commercial release in Australia in 1996. The glyphosate formulation (Roundup Ready® herbicide) approved for use by the Australian Pesticides and Veterinary Medicines Authority (APVMA) is a dry formulation that contains 690 g ae kg^{-1} of glyphosate present as a mono-ammonium salt. This herbicide may be applied over-the-top of cotton plants from planting up to, and including, the fourth true leaf (node) stage (Monsanto 2000). From the fifth true leaf stage through to canopy closure, glyphosate must be applied as either a directed or shielded spray so that there is no foliar contact. The herbicide label states that a maximum of three 1.5 kg ha^{-1} applications of the Roundup Ready® herbicide can be made in-crop with a fourth pre-harvest application at a maximum of 1.42 kg ha^{-1} per season (maximum of 5.92 kg ha^{-1} per crop). There must be at least 10 days or a minimum of two nodes of incremental growth, whichever is longer, between in-crop applications (Monsanto 2000).

Glyphosate-resistant cotton offers growers the potential to use a broad-spectrum post-emergence herbicide program. This means expanded flexibility in application, a broader spectrum of weeds to be controlled and an added convenience for conservation tillage farming (Jones and Snipes 1999). It also allows reduced dependence on soil-applied residual herbicides and increased adoption of reduced tillage practices, allowing soil and moisture conservation (Monsanto 2000). Roberts (1999) asked the question: will glyphosate-resistant cotton lead to greater use of herbicides and jeopardize the current weed management system in cotton, and therefore result in herbicide resistance? The answer lies in the management practices that producers are willing to adopt. Herbicide-resistant technology may result in the substitution or replacement of pre-emergent residuals rather than added herbicide use (Roberts 1999). If other weed management options are reduced, resistance will be the inevitable result. The success of herbicide-resistant crops will depend on the farmer’s willingness to provide a higher level of management (Roberts 1999) and increase weed management options.

2.7 POPULATION DYNAMICS OF GRASS WEEDS OF COTTON

2.7.1 *Echinochloa crus-galli* (Barnyardgrass)

Barnyardgrass originated from Europe and India and now ranges from latitude 50N to 40S, in both temperate and tropical habitations (Mitich 1990). It is a serious weed problem in 42 countries and is found in at least 27 more (Mitich 1990). The success of barnyardgrass is attributed to prolific seeding, seed dormancy, ability to grow rapidly and flower in a range of photoperiods, and relative tolerance to herbicides (Maun and Barrett 1986; Keeley and Thullen 1989). It interferes with harvesting of crops and increases labour costs (Maun and Barrett 1986). It has been reported to be most competitive under moist soil conditions (Wiese and Vandiver 1970).

2.7.1.1 Growth

Barnyardgrass prefers rich, wet soils and low to medium altitudes, but it will adapt elsewhere (Holm *et al*. 1977). The root system may extend down to 116 cm and 106 cm in lateral diameter in porous well-aerated soils (Rahn *et al*. 1968), enabling the species to withstand drought conditions. Heavy barnyardgrass infestations may remove up to 60 to 80 percent of the available nitrogen from the soil (Holm *et al*. 1977) as well as considerable amounts of other macronutrients at the expense of crops, thus reducing their yields, especially when these elements are in short supply (Vengris *et al*. 1953; Maun and Barrett 1986).

Barnyardgrass has an erect growth habit and may produce up to 15 tillers per plant. Many of the secondary and later tillers do not arise at the base of the plant but rather at any node of the stem. This contrasts with the tillering pattern of most annual grasses where tillering is restricted to the basal nodes (Norris 1991*b*).

The growth of barnyardgrass is affected by season. Barrett and Wilson (1981) found that individuals germinating in autumn yielded less total biomass and allocated a smaller proportion to roots and a larger proportion to secondary tillers and seed than individuals germinating in spring. Mitich *et al*. (1990) reported that when day lengths were 16 h, plants were twice as tall as and six times heavier than when plants were grown under day lengths of 8 to 13 h. They reasoned that plants grown under shorter day lengths pass into the flowering stage quickly and remain small in stature because vegetative development has been reduced or has ceased altogether. The rate of vegetative growth, as measured by height, appeared to be directly related to temperature, with slow extension of shoots in spring and very rapid growth in the heat of summer (Keeley and Thullen 1989).

2.7.1.2 Reproduction

Barnyardgrass, like many weed species, produces seed over a long period if growing conditions permit. Seed are shed from the plant as soon as they mature (Norris 1991). Barnyardgrass can grow and flower in photoperiods from 8 to 16 h but prefers the latter. Late summer cohorts can still produce seed, because the plant sacrifices vegetative growth for quick flowering (Mitich 1990).

The average number of seeds produced from barnyard grass varies from 1800 to more than 5000 per plant under mechanical weed control (Stevens 1932; Barrett and Wilson 1981). In trials conducted by Norris (1991*a*), plants growing at densities of less than one plant per meter of row were estimated to produce from 30 000 to over 200 000 seeds, with an average of 100 000 seeds per plant. This equated to a seed rain of about 4000 to nearly 20 000 seeds per meter from a density of one weed every 10 m of crop row. These data suggest that using economic thresholds for barnyard grass is not a sound management practice because even low densities can produce large amounts of seed. The number of seeds produced per plant decreases as plant density increases (Bosnic and Swanton 1997).

Keeley and Thullen (1989) found that with 11h day lengths, barnyardgrass produced seeds within 52 days compared to 84 days with 16 h day lengths. Therefore, decreasing day length hastens flowering. In experiments conducted by Barrett and Wilson (1981), plants grown in a glasshouse under average temperatures of 25 to 30°C took from 38 to127 days from emergence to flowering, and seed production ranged from 20 to 17 880 seeds per plant. Nutrient stress resulted in a delay in flowering, increased senescence rates and a reduction in total biomass and reproductive effort. Under long days, the delay in flowering resulted in larger vegetative biomass, lower reproductive effort and, where nutrients were limiting, inhibition of secondary tillers (Barrett and Wilson 1981).

Barnyardgrass plants are highly autogamous. The mating system involves a high degree of self-fertilization with a small amount of outcrossing by wind. This results in a high degree of homozygosity within populations and low levels of heterozygosity (Maun and Barrett 1986).

2.7.1.3 Dormancy and germination

The dormancy characteristics of barnyardgrass can be quite variable. Arai and Miyahara (1961) reported the dormancy of barnyardgrass seeds lasts about a year when seed is stored at room temperature. Barrett and Wilson (1982) stored barnyard grass seeds at room temperature for 9 and 18 months and reported that germination was higher and more consistent in the 18-month-old seed. Aria and Miyahara (1961) found that exposing seed to temperatures up to 49^{o}C and removing the seed covering, which may have an inhibitor in it, is effective in breaking dormancy. Sung *et al*. (1987) did not have success in removing the seed covering, but found scarification by sulfuric acid (H_2SO_4) broke dormancy. Some success also occurred with other chemicals such as potassium nitrate and acetone (Rahn *et al*. 1968). Alternating temperatures aid in breaking dormancy of seeds, although Matinez-Ghersa *et al*. (1997) reported that dormancy of newly dispersed seeds was broken by alternating temperature only when soil water content was sufficient to allow germination.

Maun and Barrett (1986) reported that the optimum temperature for germination of barnyardgrass seed was 5^{o}C alternating with 30^{o}C. Barnyardgrass germinates over a wide range of temperatures, from 10 to 40^{o}C (Roche and Muzik 1964; Rahn *et al*. 1968). The seeds may germinate at a rather wide soil pH range of 4.7 to 8.3 (Aria and Miyahara 1964) but the optimum for germination is around neutral (Brod 1968). Dawson and Bruns (1962) buried seeds at depths ranging from 0 to 15 cm. The maximum number of seedlings emerged from 1 to 2 cm deep. The maximum depth from which there was germination was 10 cm. Seed lying on the surface germinated but few seedlings established probably because of a lack of moisture. In saturated soils, however, seed burial caused a reduction in the rate of emergence even at 0.5 to 2 cm deep (Barrett and Wilson 1983). Horng and Leu (1978) planted seed at various depths at the beginning of November. Only 4.7 percent germinated over the first 2 months, and 25.4 percent germinated during the sixth and seventh months after planting.

Honek *et al*. (1998) studied the seasonal changes in percentage of dormant seeds of barnyardgrass taken from the field over 4 years. The proportion of seeds germinating under light conditions at a constant temperature of 25^{o}C fluctuated between 0 and 96 percent, with the majority germinating during spring and summer (Figure 2.2).

NOTE: This figure is included on page 47 of the print copy of the thesis held in the University of Adelaide Library.

Figure 2.2. The annual dormancy cycle in the percentage of seeds germinating after various periods of burial in soil. Seeds were buried in 1993 (□) or 1994 (■) and germinated at 25oC with light (Honek et al. 1998)

Increasing moisture stress delayed initial germination and slowed root and shoot elongation. The best germination occurs at about 70 to 90% field capacity of the soil (Arai and Miyahara 1963; Brod 1968). Barnyardgrass seed starting in low water content situations germinates when water content is increased up to field capacity.

Barnyardgrass seed have longevity in the soil, germinating relatively evenly over the first 6 years and tapering off to 12 years. Burnside *et al*. (1994) found that germination in the second year was low. Maun and Barrett (1986) reported that, depending on photoperiod, seed dormancy ranges from 0 to 48 months.

2.7.1.4 Crop competition

Barnyardgrass is a competitive weed in crops. In studies conducted in the United States, Keeley and Thullen (1991) found that it can cause a significant reduction in cotton yields. The effect on yield depends on time of germination and length of competition. When barnyardgrass competed with cotton for more than 3 weeks, cotton plants were shorter and cotton yields were less than in weed-free plots (Keeley and Thullen 1991). It was two times taller than cotton 6 weeks after cotton planting and remained so for the duration of the season. Barnyardgrass competing with cotton for 9 to 12 weeks reduced yields and contributed to grade reductions due to grass seed contamination in the lint (Keeley and Thullen 1991). Barnyardgrass has the ability to produce seeds within 9 weeks, which indicates the necessity of treatment within 6 weeks to prevent reproduction. In competition with cotton, barnyardgrass plants produced 350 seeds per plant at 9 weeks. This increased to 4400 seeds per plant at 12 weeks in trials. Competition for 12 weeks dramatically reduced cotton yield (Keeley and Thullen 1991).

Barnyardgrass is susceptible to shading and becomes less of a problem when it emerges after establishment of a tall and vigorous crop (Maun and Barrett 1986). Although cotton is not a good competitor early in the season, it becomes a better competitor within 6 to 9 weeks after planting. This reduces growth of barnyardgrass due to increased interception of light by the canopy of cotton (Keeley and Thullen 1991).

Bosnic and Swanton (1997) reported that seedlings that emerged at the 1 to 3 leaf maize stages cause greater maize yield reductions than later-emerging weeds. Therefore, barnyardgrass emergence time relative to maize is more critical than weed density when describing the effect of the weed on maize yield. Ten barnyardgrass seedlings emerging per meter up to the three-leaf crop stage produced 14 400 to 34 600 seeds per meter compared to only 1200 to 2800 seeds per meter from seedlings emerging after the four-leaf stage (Norris et al. 2001).

2.7.1.5 Response to weed management practices

Ogg and Dawson (1984) demonstrated that the emergence of barnyardgrass seedlings was significantly reduced by shallow tillage at monthly intervals, provided tillage started early enough in the spring. However, barnyardgrass control appears to be higher in chemical-only treatments than in tillage-only treatments. Perron and Legere (2000) found that under mechanical treatments only, seed production reached over 326 000 seeds per meter, compared to 500 seeds per meter in chemical treatments. The seasonal variation in the germination of barnyardgrass seedlings has consequences for weed management (Honek *et al*. 1998). Unlike spring and autumn cultivation, stubble breaking and pre-sowing soil preparation only brings forth sporadic germination. This absence of germination flushes undermines the use of mechanical control of barnyardgrass seedlings, which would be easier at early periods and would reduce the magnitude of seed bank replenishment (Perron and Legere 2000).

Glyphosate provides good control of barnyard grass (Lanie *et al*. 1993; Norris *et al*. 2000). However, tillered plants are more difficult to control than seedlings (Wicks and Hanson 1994). Control with increasing rates of glyphosate was better on small than large weeds (Wicks and Hanson 1994; Felton *et al*. 1990). Watering prior to spraying improves control (Felton *et al*. 1990), and herbicide treatments are less effective where the soil water levels are below field capacity (Ahmadi *et al*. 1980). Lanie *et al*. (1993) also found that the addition of residual herbicides to glyphosate did not improve control. However, the addition of residual herbicides to paraquat did increase control of barnyardgrass.

Barnyardgrass resistance to triazines has been reported in Canada and Europe (Maun and Barrett 1986; Lopez Martinez *et al*. 1997; Heap 2006). Resistance to atrazine began to appear after four consecutive years of treatment and thereupon increased steadily with each year of exposure. Propanil-resistant barnyardgrass has been identified in the United States in Arkansas and Texas in rice fields (Smith and Baltazar 1993).

Early season weed control is very important to reduce barnyardgrass weed seed production and thereby minimize long-term seed banks (Bosnic and Swanton 1997). Use of early cultivation or soil disturbance will encourage some germination of the weed (Roberts 1984; Honek *et al*. 1988), and residual herbicides such as metolachlor do provide some control (Lanie *et al*. 1993). These can be combined with glyphosate treatments and crop competition via shading for an integrated approach to control.

2.7.2 *Urochloa panicoides* (Liverseed grass)

Liverseed grass is among the most important weeds found in fallow in northern NSW and southern QLD (Felton *et al*. 1990; Felton *et al*. 1994). Liverseed grass is a pasture species in the savannas of Botswana (Veenendaal *et al*. 1995) and is also found in India (Cunningham *et al*. 1981). In Australia, it is also found along roadsides, stockyards and other disturbed areas. It grows on soils ranging from sands through to clays (Cunningham *et al*. 1981).

2.7.2.1 Growth and reproduction

Liverseed grass is an annual tufted to semi-prostrate grass which prefers moist soil and shade (Cunningham *et al*. 1981; Veenendaal *et al*. 1996). Mature plants can grow to 75 cm tall (Cunningham *et al*. 1981), and the stems can sometimes take root where nodes of tillers touch the ground (Wilson *et al*. 1995). In the mature plant, 30% of total biomass consists of flowers and seeds with seed alone comprising 4% of total biomass (Veenendaal *et al*. 1996). The inflorescence comprises a panicle up to 7.5cm long with 2 to 7 erect spikes (Cunningham *et al*. 1981). Veenendaal *et al*. (1996) reported that seed production can vary from 200 to 2400 seeds per meter, and that subsequent emergence was 4-984 seedlings per meter due to the existence of a large seed bank. Liverseed grass flowers from summer through to autumn (Cunningham *et al*. 1981).

2.7.2.2 Germination

Veenendaal *et al*. (1995) reported that liverseed grass showed a peak germination in spring, and germinated relatively soon after rainfall. Seedling survival was relatively high with 20% survival after 100 days. The survival of liverseed grass appears to be enhanced by shading (Veenendaal *et al*. 1995). Seedlings can emerge from a depth of 5 cm. Veenendaal *et al*. (1995) reported that 50% of buried seeds germinated from a depth of 1 cm, with 15% of seedlings germinating from 2 and 3 cm deep and only 5% from 5 cm.

2.7.2.3 Response to weed management practices

Liverseed grass is an important weed in cropping situations. Its growth in summer fallows is a major problem because it rapidly depletes soil moisture and nutrients (Adkins *et al*. 1998). It is highly competitive with crops such as cotton. Roberts (1999) reported a 61% yield reduction where Liverseed grass was uncontrolled. In a survey by Felton *et al*. (1994) Liverseed grass was present in wheat stubble and grain sorghum whether or not atrazine was use, regardless of tillage.

Liverseed grass is prone to moisture stress, which affects the efficacy of herbicide applications (Adkins *et al*. 1998). Water stress reduces the leaf area, which in turn leads to a reduction in the amount of herbicide assimilated by the plant (Adkins *et al*. 1998). Felton *et al*. (1990) found that control with 450g ha^{-1} of glyphosate was poor and that better control occurred with 625 to 900g ha^{-1}. Roberts (1999) examined the effect of glyphosate and residual herbicides on liverseed grass. He reported that the residual herbicides controlled a small number of plants initially but proved effective in preventing a second germination that occurred in the glyphosate-only treatments after rainfall. While the glyphosate-only treatment was able to control existing seedlings, there was no capacity to control plants that germinated after rainfall.

In 1996 liverseed grass first evolved resistance to atrazine in Queensland (Adkins *et al*. 1997), however as yet the mechanism conferring resistance has not been determined (Heap 2006).

2.8 RESISTANCE MODELING

The use of computer models as aids for management decisions is becoming an important tool in agriculture. Models have been integrated into decision making software or decision support systems such as CottonLogic and WHEATMAN. Such software incorporates measured data into simulations, which can predict outcomes with different treatments applied.

In the context of weed science, Forcella *et al*. (1996) developed the WEEDSIM bioeconomic decision aid for the management of weeds in corn and soybean. This integrates the essential aspects of weed and crop ecology, weed control and management economics. There are a number of computer-based decision support systems for use in weed management. These include SOYHERB, which provides recommendations on herbicide treatments (Mortensen and Coble 1991). Jones and Medd (1997) developed a dynamic programming model to examine the impact of a range of management strategies for the control of wild oats in wheat. This model provided a means of determining the optimal combination of strategies over time for various initial values of the seed bank.

2.8.1 Modeling herbicide resistance

Herbicide resistance and pressure for environmental sustainability are forcing farmers to carefully consider options for weed control. Farmers face a number of difficulties in their decision making about weed management options. Strategies must be evaluated over the long term, and not just for a single year. The long-term impacts of multiple control options are difficult to predict, and the impacts of individual treatments within an integrated strategy may be difficult to interpret from field observations (Pannell *et al*. 2003). Computer simulation models may be able to generate the data that are required to answer questions in the short term. By combining knowledge of production ecology with the economic principles of weed control into a model, simulations can be run that may help to analyze the performance of alternative crop husbandry options under a range of conditions (De Buck *et al*. 1999).

Modeling may also be a useful tool for resistance management. The complexity of the biological processes that influences the development of herbicide resistance requires a research approach that focuses on the interaction between the plant's life processes and population genetics. Models can assist in better understanding these complex biological processes while providing a tool to evaluate the usefulness of different management tactics (Maxwell *et al*. 1990). The specific aspects of each herbicide, weed species and particular resistance characteristics will determine the effectiveness of management strategies. Often these specifics are not known, requiring a certain amount of extrapolation to make management recommendations (Christoffers 1999). Modeling can discriminate between different strategies and so determine which strategy can prevent resistance in a majority of real situations (Cavan *et al*. 2000). Jasienuik *et al*. (1996) reviewed the use of models on population genetic factors influencing the evolution of herbicide resistance in weed populations. They found that with the appropriate assumptions, models could be invaluable in assessing the relative effectiveness of various weed management practices to avoid or delay the occurrence of herbicide resistance in weed populations.

Gressel and Segel (1978) explored the sensitivities among the major factors affecting the evolution of herbicide resistance, including fitness, selection pressure and soil seed bank dynamics. Increased understanding of how these processes influence resistance development enables prescriptive management recommendations to reduce the risk of resistance evolution and spread. Later, Gressel and Segel (1990) adapted their earlier model in order to understand the effectiveness of herbicide rotations and mixtures to prevent/delay resistance evolution.

Maxwell *et al*. (1990) developed a model combining the demographic processes of an annual weed and the processes of seed and pollen migration. The demographics component of the model utilized an inheritance submodel based on the Hardy-Weinberg principle of gene segregation. The authors coupled the processes of gene flow to the ecological fitness characters of susceptible and resistant biotypes which were demonstrated as key driving forces in the evolution of resistance (Diggle and Neve 2001).

Cavan *et al*. (2000) developed a model to investigate strategies to prevent resistance in black-grass (*Alopecurus myosuroides*). The authors determined that resistance could be delayed indefinitely if three herbicides, each with a different mode of action, are rotated and a 95% kill rate is maintained throughout.

Gardner *et al.* (1998) developed a framework that modeled the simultaneous evolution of quantitative and major monogene resistances. Outputs from their simulations promoted a strategy to use a revolving rotation of high and low dose rates to delay the development of both types of resistance.

Pannell *et al.* (2003) developed the RIM (Ryegrass Integrated Management) model, which represents a comprehensive set of weed control treatments, including both herbicide and non-herbicide options. The model evaluates the impacts of reducing herbicide availability for the selection of weed control practices to assess their economic consequences. RIM is useful for farmers, scientists, agronomists and industry in understanding the management of herbicide-resistant ryegrass as it also involves economic aspects.

In order to examine the effect of the pattern of herbicide use on the development of resistance Diggle *et al.* (2003) developed a model describing the effect on resistance of using herbicides with different modes of action in combination. The simulations run using this model highlighted the importance of keeping weed numbers low. They demonstrated that where resistance genes occur at low frequencies in small populations, it is possible that these genes will become extinct. Therefore, management practices that segregate weed populations into smaller, genetically isolated units and can resist gene movement may result in a lower incidence of herbicide resistance. The authors were able to conclude from the model that herbicides used in combination are significantly more effective at delaying resistance than using them in rotation, provided both herbicides are effective.

Neve *et al.* (2003) adapted the model developed by Diggle *et al.* (2003) to simulate the evolution of glyphosate resistance in rigid ryegrass based on empirical data of a glyphosate-resistant population. These simulations showed that timing of herbicide application in relation to germination could influence the rate of resistance evolution. With applications of glyphosate close to the commencement of the growing season exposing less of the population to glyphosate reduced the selection pressure for resistance. Neve *et al*. (2003) also attempted to gain an understanding as to why the evolution of glyphosate resistance in rigid ryegrass had been considerably slower than resistance evolution to other herbicide modes of action. They suggested the reason was related to lower initial homozygous resistant gene frequencies, fitness penalties and past patterns of herbicide use.

Neve *et al*. (2003*a*) developed a herbicide resistance model predicting rates and probabilities of glyphosate and paraquat resistance evolution under different use strategies. By simulating population dynamics and genetics they modeled the impacts of tillage systems and the effects that the introduction of glyphosate-resistant canola would have on glyphosate use. The model predicted that glyphosate use would increase with glyphosate tolerant canola and therefore the percentage of resistant ryegrass populations would also increase.

2.9 CONCLUSIONS

Weeds persist in production systems through tolerance to weed management practices such as herbicides or tillage and due to growth types and life cycles within that production system. The key to managing or preventing species shifts is to anticipate which weeds will survive specific management systems (Madsen and Streibig 2002).

Herbicide-resistant crops provide a powerful new tool for the management of weeds and offer greater flexibility and new solutions for difficult weed problems (Burnside 1992). They also provide new uses for existing herbicides (Roush 2002). The introduction of glyphosate-resistant cotton in Australia is likely to alter conventional weed management practices and also the weed spectrum that exists in crop fields. Glyphosate is widely used as a post-emergent broad spectrum herbicide in the cotton industry, especially in fallow situations. Glyphosate-resistant cotton increases the opportunities to use glyphosate in the cotton cropping phase of a cotton system. There are currently no resistant weeds in cotton systems in Australia; however, glyphosate-resistant cotton may lead to greater use of glyphosate and place the entire system at jeopardy due to herbicide resistance (Roberts 1999).

Several models have been developed with regard to resistance evolution in other cropping systems. It is important that the effects of management practices, particularly in glyphosate-resistant cotton, be examined for their influence on weed population dynamics and resistance selection in cotton systems in Australia. Models can help do this and may provide the ability to predict the likelihood and time frame for resistance evolution under different strategies. This will enable the possible altering of strategies for weed management to reduce the risks of resistance evolution.

CHAPTER 3

GROWER SURVEY – WEED MANAGEMENT PRACTICES IN GLYPHOSATE-RESISTANT AND CONVENTIONAL COTTON FIELDS IN AUSTRALIA

3.1 INTRODUCTION

Glyphosate-resistant (Roundup Ready®) cotton has been adopted rapidly throughout the Australian cotton industry since its introduction in the 2000/01 season. In 2005/06, glyphosate-resistant cotton accounted for almost 70 percent of total cotton area (G. Constable, CSIRO, pers. comm.). The reasons for this rapid adoption include the wide spectrum of weeds susceptible to glyphosate and reduced cotton seedling damage commonly associated with the use of pre-emergence and pre-plant herbicides (Charles *et al*. 1995). Importantly, the adoption of this technology promotes the use of conservation tillage practices and allows growers the option of using a broad-spectrum, post-emergent herbicide in-crop (Jones and Snipes 1999; Faircloth *et al*. 2001).

The Australian cotton industry traditionally uses an integrated approach to weed management (Charles 1991). This integrated system, which has evolved over time, includes use of herbicides with different modes of action, shielded applications of glyphosate and other herbicides in the inter-row furrows as well as tillage and hand hoeing for weed control (Roberts 1998*a*). This integrated weed management strategy may be at risk if cotton growers pursue a management strategy based predominantly on glyphosate. With the continued development of conservation tillage practices and the high cost of hand-hoeing, a number of these alternative management strategies may be relinquished in favour of more cost effective herbicide applications, particularly glyphosate.

This reduction in alternative management strategies, combined with increased selection pressure due to an increased number of glyphosate applications, has the potential to lead to herbicide resistance. Charles *et al*. (1995) have determined that 14 of the 20 most common weeds found on cotton farms are either favoured by reduced cultivation, are tolerant of glyphosate, or both. Herbicide resistance evolves due to an increase in the numbers of individuals carrying a resistance allele as a result of increased selection pressure caused by repeated applications of the same herbicide (Betts *et al*. 1991; Jasieniuk *et al*. 1996) and thus has appeared where one or a few herbicides have been used repeatedly in weed management (Preston and Rieger 2000). The evolution of glyphosate-resistant *Conyza canadensis* in glyphosate-resistant soybeans in the United States, within three years of continuous glyphosate use as the major form of weed control (VanGessel 2001), highlights the risk of glyphosate resistance developing in a glyphosate-resistant cropping system.

The purpose of this survey was to determine how weed management practices were changing with the introduction of glyphosate-resistant cotton. Interviews were conducted with growers who planted both glyphosate-resistant and conventional cotton in their fields so that comparisons could be made on weed species composition, weed management practices, glyphosate dose rates and application timings. This allowed the effects of the introduction of glyphosate-resistant technology on the weed spectrum and glyphosate use to be quantified. Information was also obtained regarding grower perceptions on the cost effectiveness of glyphosate-resistant systems, effectiveness of weed control and the possible implications of improved glyphosate-resistant technology on current weed management in glyphosate-resistant cotton.

3.2 MATERIALS AND METHODS

The survey targeted 40 growers, comprising 10 growers in each of the four major cotton-growing regions in March 2003. These were the Darling Downs and MacIntyre regions in Queensland and the Gwydir and Lower Namoi regions in New South Wales. These four regions contribute up to 70 percent of all irrigated cotton grown in Australia (Dowling 2003). The area of cotton, percentage of glyphosate tolerant cotton and the number of years glyphosate-resistant cotton had been grown are detailed in Table 3.1.

3.2.1 Experimental procedure

The property owner, manager or agronomist responsible for weed management (termed grower hereafter) was interviewed. Growers were asked to list their main weeds in conventional fields and in fields where they had grown glyphosate-resistant cotton for one or more years. Growers were also asked to indicate weeds for which they obtained improved control using the glyphosate-resistant technology, weeds that were not controlled as effectively in glyphosate-resistant cotton and weeds that were not being controlled in either system. Growers were also asked to nominate weed problems that had influenced their decision to use glyphosate-resistant cotton in particular fields.

Table 3.1. Area of cotton grown, percentage of glyphosate-resistant cotton planted and average number of years glyphosate-resistant cotton has been grown, for the surveyed growers in each of the regions.

	Area of cotton grown per farm (ha)			Percentage area of glyphosate-resisant cotton grown	Number of years glyphosate-resistant cotton grown
Region	**Mean**	**Highest**	**Lowest**		
Darling Downs	338	1420	83	36	3.0
Gwydir	917	3040	270	47	2.3
Lower Namoi	637	2200	150	52	2.1
MacIntyre	643	980	80	43	2.0
Mean	633.8	1910	145.8	44.5	2.4

The herbicide use patterns in glyphosate-resistant and conventional fields, including quantity, application methods and timings, were determined from growers' records. Growers were also asked for their opinion on how the introduction of enhanced glyphosate-resistant cotton, capable of tolerating over-the-top applications of glyphosate throughout the life of the crop, might further influence their weed management practices compared with the current glyphosate-resistant cotton technology.

3.2.2 Statistical analysis

Data on crop rotations, weed species occurrence and prevalence, weed management, herbicide use, opinions on cost effectiveness of the current glyphosate-resistant technology and the potential of enhanced glyphosate-resistant technology were tabulated. Quantitative data on herbicide use were analysed using ANOVA (GenStat 7th edn., VSN International, Hertz, UK) to test whether there were significant differences between regions and between glyphosate tolerant and conventional crops. For this analysis, glyphosate use in the rotation was calculated by dividing the total amount of glyphosate used in the rotation by the number of years of the rotation. Where a grower was uncertain of glyphosate usage in fallow, an assumption of two fallow sprays of 450 g ha^{-1} was made. Glyphosate use in the cotton crops was calculated for the period from one month prior to planting until the cotton was harvested.

3.3 RESULTS

3.3.1 Crop rotations

In general, growers adopted the same crop rotations regardless of the type of cotton grown (Table 3.2). The rotations adopted ranged from cotton only, to combinations of summer and winter cereals and pulses. On the Darling Downs, 80 percent of growers used summer and winter cereals in rotation with pulses. In the Gwydir region, the use winter of cereals was more popular, with 60 percent of growers using this rotation. A summer cereal rotation was employed by half of the growers surveyed in the Lower Namoi region, while half of the growers in the MacIntyre region grew continuous cotton.

Table 3.2. Crop rotations used in conjunction with conventional and glyphosate-resistant cotton across all regions surveyed.

Rotations	Other crops used in rotations	No. of growers using rotation Glyphosate-resistant fields	Conventional fields
Cotton only	None	9	9
Cotton and pulses	Chickpeas, vetch	5	5
Cotton and winter cereals	Wheat, barley	11	12
Cotton and summer cereals	Sorghum	5	5
Cotton, summer and winter cereals	Sorghum, maize, wheat, barley	13	11
Cotton, winter cereals and pulses	Wheat, faba beans	1	1
Cotton, summer cereals and pulses	Sorghum, mungbeans	1	1
Cotton, summer and winter cereals and pulses	Sorghum, maize, wheat, barley, chickpeas	0	1

3.3.2 Flexibility and cost effectiveness of glyphosate-resistant cotton

Over 80 percent of growers indicated that the flexibility of management associated with growing glyphosate-resistant cotton was a major benefit of the crop (Table 3.3). The ability to use glyphosate over-the-top provides an additional weed management option. This benefit was particularly important in fields with high weed pressure. In relation to cost effectiveness now and in the future, over half of growers surveyed stated that glyphosate-resistant cotton was cost effective to use despite the requirement to pay a licence fee, and would use it in the future. Some growers found that the cost effectiveness depended on seasonal conditions and was less in dry years.

Table 3.3. Grower perceptions of the flexibility and cost effectiveness of glyphosate-resistant cotton in March 2003.

	Growers' response (%)		
Question	**Yes**	**No**	**Undecided**
Did increased flexibility offered by glyphosate-resistant cotton influence your decision to grow the crop?	88	8	5
Is glyphosate-resistant cotton cost effective now?	65	23	13
Will glyphosate-resistant cotton continue to be cost effective?	58	20	23
Will you increase the area of glyphosate-resistant cotton planted in the future?	53	28	20

3.3.3 Weed species and prevalence

Growers listed 25 important weeds in their glyphosate-resistant fields and 24 in their conventional fields. Of these, 10 weeds were present on more than 20 percent of farms across all regions surveyed (Table 3.4). These weeds were the same in glyphosate-resistant and conventional fields, although the order of importance differed slightly. *Cyperus rotundus* L., for example, ranked as the fourth most common weed in glyphosate-resistant fields, as opposed to the seventh most common weed in conventional fields.

Table 3.4. The 10 most common weeds in conventional and glyphosate-resistant cotton fields as reported by growers.

Weed species	Presence of weed in conventional fields (%)	Presence of weed in glyphosate-resistant fields (%)
Hibiscus trionum spp.	70	80
Xanthium occidentale	63	65
Datura stramonium	55	55
Echinochloa spp.	43	48
Ipomea lonchophylla	35	38
Tribulus terrestris	30	35
Cyperus rotundus	28	50
Sesbania cannabina	25	28
Xanthium spinosum	25	25
Polymeria pusilla	20	23

Growers stated that the reason for the greater presence of certain weeds in glyphosate-resistant fields lay in them using the glyphosate-resistant technology on fields with higher weed pressure and where the weeds were perceived to be more difficult to control. The higher percentage of glyphosate-resistant fields with *Cyperus rotundus* and *Tribulus terrestris* L. are prime examples of this. *Hibiscus trionum* spp., *Xanthium* spp., *Datura stramonium* L., *Echinochloa* spp., *Ipomea lonchophylla* J.M.Black, *Cyperus rotundus*, and *Tribulus terrestris* were common to all regions and fields. *Polymeria pusilla* R. Br. was common in both conventional and glyphosate-resistant fields in the Darling Downs, MacIntyre and Lower Namoi regions. *Sesbania cannabina* (Retz.) Pers. was common in both fields in the Gwydir, MacIntyre and Lower Namoi regions. *Hibiscus trionum*, the most common weed found in irrigated cotton fields, was also the most common weed found in dryland paddocks surveyed by Walker *et al*. (2005). Other weeds, such as *Xanthium occidentale* Bertol. and *Datura stramonium*, appear to be greater problems in irrigated fields. Weeds common to both situations were *Tribulus terrestris*, *Ipomea lonchophylla* and *Echinochloa* spp. The weed spectrum reported in this survey was different than that reported by Charles (1991). *Xanthium occidentale* now affects a larger percentage of properties. *Hibiscus trionum* has also increased in importance since the survey, where it was ranked sixth. *Echinochloa* spp., ranked twelfth in the same survey, has also become more prominent.

Half of the growers said no weeds were more prevalent after using glyphosate-resistant cotton; however, only 38 percent of growers felt they achieved better control on all weeds. It may be too early to observe weed species shifts as glyphosate tolerant cotton has only been used for three seasons.

Table 3.5. Weeds that influenced the grower's decision to grow glyphosate-resistant cotton, and perception of weed prevalence and control in glyphosate-resistant and conventional cotton fields.

Weed species	**Weed influenced decision to grow glyphosate-resistant cotton (%)**	**Weed more prevalent (%)**	**Weed better controlled (%)**	**Inadequate control in either crop (%)**
All species			38	
No species		50	13	30
Cyperus rotundus	38		25	15
Hibiscus trionum	18	15	15	
Glyphosate tolerant cotton volunteers		20		10
Echinochloa spp.	10		8	
Datura stramonium	8		8	
Xanthium occidentale	10		5	
Physalis minima			5	5
Ipomea lonchophylla	8			13
Sesbania cannabina		5		
Polymeria pusilla				5
Rhyncosia minima				5
Solanum spp.	8			
Xanthium spinosum	5			

Most growers (75 percent) surveyed reported that they had adopted glyphosate-resistant cotton as a result of specific weed problems. The most common of these, as indicated in Table 3.5, was *Cyperus rotundus*, with 38 percent of growers adopting the technology because of this weed.

There are few management options for the control of this weed, and glyphosate is considered the main herbicide for its control (Bariuan *et al*. 1999). Approximately two-thirds of these growers felt they gained better control of *Cyperus rotundus*, while the rest reported control was inadequate.

3.3.4 Weed management

There was a 21 percent decline in the number of growers using a full pre-emergence residual herbicide program involving the application of residual herbicides both before and at planting (Table 3.6) following the adoption of glyphosate-resistant cotton. Some of these growers had moved to a reduced residual herbicide program in their glyphosate-resistant cotton fields. These growers were applying residual herbicides prior to or at planting only rather than at both times.

Other chemical weed control options, such as post-emergence herbicides and lay-by residual applications, dropped only slightly in frequency of use, with the majority of growers choosing to use these methods in addition to glyphosate. There was a slight decrease in the use of glyphosate as a knockdown herbicide in glyphosate-resistant crops and an increase in the use of alternative knockdown herbicides. Mechanical weed control options, such as tillage and hand-hoeing, were marginally reduced in glyphosate-resistant cotton fields, with the main effect being a reduction in the number of cultivation passes.

Table 3.6. Percentage of growers adopting various weed management practices in glyphosate-resistant and conventional cotton fields.

Weed management practice	Growers using in conventional cotton (%)	Growers using in glyphosate tolerant cotton (%)
Full pre-emergence residual herbicide	95	74
Reduced pre-emergence program		13
Pre-emergence knockdown herbicide		
Glyphosate	36	31
Other	8	13
Roundup Ready[A] over-the-top	0	100
Post-emergence herbicide (not glyphosate)	21	13
Lay-by residual herbicide	67	62
Shielded spray		
Glyphosate	18	59
Other	0	5
Spot Spraying	10	5
Inter-row cultivations	92	87
Hand hoeing	80	72

[A] Roundup Ready® herbicide is a specific formulation of glyphosate 690g kg^{-1} registered for over-the-top use in glyphosate-resistant cotton.

3.3.5 Herbicide mode of action

Information on the range of herbicides used in cotton fields was obtained. These herbicides were grouped according to the Crop Life Australia herbicide modes of action (Crop Life Australia 2006). Herbicides from Groups C (Prometryn, fluometuron and diuron) and D (pendimethalin and trifluralin) were the main residual herbicides used in cotton (Charles *et al*. 1995). There was a 15 percent reduction in the use of Group C and a 18 percent reduction in the use of Group D chemicals in glyphosate-resistant cotton crops (Table 3.7). There was also a substantial reduction in the use of Group B selective broadleaf herbicides, such as pyrithiobac-sodium and trifloxysulfuron, but an increase in the use of Group L (paraquat and diquat) and M (glyphosate) herbicides.

Table 3.7. Herbicide Modes of Action used in conventional and glyphosate-resistant cotton fields.

Mode of Action Group[A]	Growers using Mode of Action group in conventional fields (%)	Growers using Mode of Action Group in glyphosate-resistant fields (%)
A (ACCase inhibitors)	5	3
B (ALS inhibitors)	25	15
C (PSII inhibitors)	95	80
D (Microtubule assembly inhibitors)	78	60
I (Plant cell growth disruptors)	13	10
K (Other modes of action)	8	3
L (PSI disruptors)	8	13
M (EPSP synthase inhibitors)	68	100

[A] Australian herbicide mode of action labeling system.

3.3.6 Herbicide use

Quantitative data on herbicide use were obtained from growers' records. As expected, glyphosate use was higher in glyphosate-resistant fields than in fields planted with conventional cotton. Glyphosate use in glyphosate-resistant cotton fields ranged from 2.3 to 3.2 kg ae ha^{-1} across the four regions (Table 3.8). This compares to around 0.5 kg ha^{-1} in conventional cotton crops. When the whole rotation was considered, glyphosate use averaged 2.5 kg ha^{-1} per annum for rotations containing glyphosate-resistant cotton and 0.9 kg ha^{-1} per annum for rotations with conventional cotton.

Table 3.8. Glyphosate use in the cotton crops and in the full rotation across regions.

Region	Full rotation[A*]		Cotton crop[B†]	
	Glyphopsate use in glyphosate-resistant cotton (g ae ha^{-1})	**Glyphosate use in conventional cotton (g ae ha^{-1})**	**Glyphosate use in glyphosate-resistant cotton (g ae ha^{-1})**	**Glyphosate use in conventional cotton (g ae ha^{-1})**
Darling Downs	2785	707	3175	493
Gwydir	2673	999	2822	585
Lower Namoi	2115	919	2612	791
MacIntyre	2387	974	2348	485
Average	2490	900	2739	589

[A] Average per annum over the whole rotation (2 – 4 years).

[B] Average from 1 month prior to planting to picking (approx. 9 months).

*In full rotation, l.s.d. = 392 ($P = 0.05$).

[†] In cotton crop, l.s.d. = 362 ($P = 0.05$).

The increase in glyphosate use in glyphosate-resistant cotton fields led to a reduction in the use of herbicides other than glyphosate. However, this reduction was relatively slight, with an average reduction from 3.38 to 2.55 kg a.i. ha^{-1} (Table 3.9). A significant reduction (more than 50 percent) in other herbicides used did occur in glyphosate-resistant cotton fields on the Darling Downs, due to a number of growers using glyphosate as the only herbicide. Growers who had poor control of later-germinating weeds stated they would use residual herbicides in the future. However, those with lower weed pressure were satisfied with the level of weed control obtained from a glyphosate-only program.

Table 3.9. Use of herbicides other than glyphosate in glyphosate-resistant and conventional cotton fields.

Region	Herbicides other than glyphosate used in conventional cotton (kg a.i. ha^{-1})	Herbicides other than glyphosate used in glyphosate-resistant cotton (kg a.i. ha^{-1})
Darling Downs	2.96	1.30[A]
Gwydir	3.93	3.38
MacIntyre	3.13	2.38
Namoi	3.52	3.15
Average	3.38	2.55

[A] Significant ($P < 0.05$), l.s.d. = 1.347.

3.3.7 Possible changes to weed management with enhanced glyphosate-resistant technology

One of the problems with the current glyphosate-resistant cotton varieties is that over-the-top glyphosate applications cannot be made past the four-leaf stage, otherwise there is a risk of a reduction in yield (Pline *et al*. 2002). Enhanced glyphosate-resistant cotton technology allowing a wider application window has been developed for use in the United States and in Australia (May *et al*. 2004). Growers in this survey indicated they would readily adopt such improved glyphosate-resistant technology (Table 3.10). Nearly half of growers surveyed considered they would increase the number of glyphosate applications to these crops. The remaining growers said they would rather try to spread applications out to achieve the greatest benefit of weed control.

Over 50 percent of growers stated they would reduce or eliminate hand hoeing due to its high cost. However, some growers thought that selective hand hoeing might still be necessary. Some growers (30 percent) said they would reduce the amount of cultivation, although in irrigated paddocks cultivation would still be required to obtain adequate water flow along the furrows. The use residual herbicides is likely to decline with the introduction of enhanced glyphosate-resistant cotton; however, a number of growers still considered residual herbicides to be an important weed management option.

Table 3.10. Grower perceptions on possible changes in weed management practices with the introduction of enhanced glyphosate-resistant cotton.

Weed management practice	Change in weed management practices (%)				
	Darling Downs	Gwydir	Lower Namoi	MacIntyre	Mean
Pre-emergence residual herbicides	-10	-40	-70	-10	-32.5
Post-emergence selective sprays	-10	0	-40	0	-12.5
Lay-by applications	-10	-20	-10	-30	-17.5
Cultivation	-40	-20	-10	-50	-30
Chipping	0	-50	-90	-70	-52.5
Increase in Roundup Ready[A] applications	10	20	80	80	47.5
Mean[B]	-13	-25	-50	-40	-32

[A] Roundup Ready® herbicide is a specific formulation of glyphosate 690g kg^{-1} registered for over-the-top use in glyphosate tolerant cotton.

[B] In this table the increase in Roundup Ready® applications has been considered a negative IWM practice in terms of calculating the average change in IWM practices in Roundup Ready® cotton.

3.4 DISCUSSION

At this early stage of adoption, some changes in weed management practices are already apparent with the introduction of glyphosate-resistant cotton. The flexibility of an added weed management option for the purpose of targeting weeds had a more substantial influence on the decision to grow glyphosate-resistant cotton than did the rotation used by the grower. In nearly all cases, growers stated that the rotation they used depended on water availability. As the survey was conducted among growers of irrigated cotton, irrigation allowed a wider range of crop rotations than those available in dryland situations. In irrigated systems, the most common rotation involved cotton, winter cereals and summer crops. This compares to non-irrigated or dryland cotton where the most common rotation is cotton and winter cereals (Walker *et al*. 2005).

Also in irrigated systems, more than twice as many growers opt for cotton-only rotations than in dryland situations (Walker *et al*. 2005).

The impact of glyphosate-resistant cotton on crop rotations in Australia is in contrast to the situation in Canada, where herbicide-resistant canola was seen by over half the growers surveyed to provide greater flexibility in crop rotations due to better weed control (Serecon and Koch Paul 2001). Similarly, triazine-tolerant canola grown in Australia allows better control of cruciferous weeds such as *Raphanus raphanistrum* L. and has significantly altered crop rotations (Stanley 2006).

There has also been a relatively rapid adoption of the technology among cotton growers, and most growers (53 percent) stated they would increase the area planted to glyphosate-resistant cotton on their farm in the future. However, other factors, such as inadequate fusarium (*Fusarium oxysporum*) tolerance in currently available glyphosate-resistant cotton varieties, will have an effect on the area planted.

The survey conducted by Doyle *et al*. (2003) also concluded that glyphosate-resistant cotton provided an effective tool for targeting problem weeds. *Cyperus rotundus* and *Hibiscus trionum* were examples of this. Some growers achieved good control of *Hibiscus trionum* while others had problems with later-germinating individuals when residual herbicides were not applied. *Ipomea* spp. and *Polymeria* spp. were not controlled adequately in either type of cotton.

Growers stated almost unanimously that the window for over-the-top application of glyphosate was too narrow. Those who substituted glyphosate for other weed control methods, in particular residual applications applied mid-season near the base of the cotton plant (lay-by applications), had problems with weeds germinating later in the season as a result of rainfall and irrigation events. This was also an issue raised by Doyle *et al*. (2003). Some growers found that using glyphosate, a broad spectrum herbicide, over-the-top gave them better control of early season weeds and those in the plant line.

There was a tendency to use selective hand-hoeing to control escapes instead of employing it as a major source of weed control due to better early season weed control. Inter-row cultivation was still seen as important as a means of ensuring adequate flow of irrigation water along the furrows. Similarly, in Canada, the adoption of herbicide-resistant canola resulted in only slight reductions in tillage (Serecon and Koch Paul 2001) as the number of tillage operations was reduced by one.

A reduction in residual herbicides applied was evident, with 95 percent of growers using the normal pre-emergence residual program in conventional cotton compared to 74 percent in glyphosate-resistant cotton. Doyle *et al*. (2003) reported similar results with 27 percent of growers adopting a reduced residual program in glyphosate-resistant cotton. The availability of effective post-emergence herbicide options is likely to lead to a reduction in the less reliable and more difficult to apply soil residual herbicides. Canola growers in Canada reduced their use of pre-emergence herbicides in herbicide-resistant canola (Serecon and Koch Paul 2001), as did some glyphosate-resistant soybean growers in the United States (Lin *et al*. 2001). However, glyphosate-only herbicide programs have resulted in the evolution of glyphosate-resistant *Conyza canadensis* (L.) Cronq. in glyphosate-resistant soybeans (VanGessel 2001).

The main concern with the introduction of glyphosate-resistant technology is that glyphosate applications will be substituted for other weed control methods, eventually leading to herbicide resistance (Roberts 1999). For example, an increase in glyphosate use has also been observed in glyphosate-resistant soybeans, where glyphosate use increased from 0.25 kg ha^{-1} to 0.66 kg ha^{-1} (Lin *et al*. 2001). However, it is the application frequency that is likely to result in an increased resistance threat rather than the total amount of active ingredient used (Preston and Roush 1998). Glyphosate use in glyphosate-resistant cotton in Australia has increased substantially without a dramatic reduction in other weed management tactics. Importantly, it had added only two additional application timings (from emergence to four true leaves) because tolerance in cotton is not absolute. In glyphosate-resistant canola, glyphosate use per hectare rose only marginally, but the proportion of growers using glyphosate in canola increased from 74 percent to 100 percent (Serecon and Koch Paul 2001). This was also evident in cotton in Australia as the percent of growers using glyphosate in-crop rose from 68 percent in conventional fields to 100 percent in glyphosate-resistant fields.

The introduction of enhanced glyphosate-resistant technology in cotton is likely to have an effect on weed management, with growers indicating that their integrated weed management practices could be reduced overall by approximately 30 percent. The size of these changes will depend on factors such as grower understanding of the technology and weed populations. It is highly likely, as was the case with the current glyphosate-resistant cotton, that growers in the first few years will use the technology in a conservative manner, maintaining a number of alternative weed management practices. However, there is a danger that in subsequent years this integrated approach could be abandoned for glyphosate-only management. If this were to take place, the threat of glyphosate resistance occurring in Australian cotton fields would increase dramatically.

Overall, three years after the introduction of glyphosate-resistant cotton, Australian growers appear to be using the technology as an addition, rather than substitution, to the conventional integrated weed management program, thus not increasing the risk of the development of glyphosate resistance.

CHAPTER 4

POPULATION DYNAMICS OF BARNYARDGRASS AND LIVERSEED GRASS UNDER A RANGE OF WEED MANAGEMENT REGIMES IN A GLYPHOSATE-RESISTANT COTTON SYSTEM.

4.1 INTRODUCTION

Before the introduction of glyphosate-resistant cotton, weed control systems in cotton fields in Australia involved high rates of residual herbicides, frequent inter-row cultivation and hand-hoeing (Charles *et al*. 1995). This system had the makings of an integrated weed management (IWM) system. However, the use of cultivation and hand-hoeing in-crop are expensive and therefore are not seen as attractive solutions to weed management (Roberts 1998*b*). Also, the shift towards permanent beds, reduced cultivation and stubble retention by some growers is putting increasing pressure on herbicides to provide all the weed control (Roberts 1998*b*). In addition, when heavy rainfall washes residual herbicides into the seed zone, damage to cotton seedlings results. Therefore, there is a desire to reduce reliance on these herbicides (Charles *et al*. 1995).

Glyphosate resistance in cotton provides growers with another weed control option within the crop, allowing the use of a broad spectrum, post-emergent herbicide (Jones and Snipes 1999, Faircloth *et al*. 2001). Glyphosate is recognized as an environmentally benign herbicide that is effective on annual and perennial grasses, broadleaf weeds and sedges (Culpepper and York 1998). Factors such as these may result in the substitution of glyphosate for other weed control options. This is likely to increase the threat of resistance to glyphosate evolving (Roberts 1999), as resistance results from the use of one or few herbicides persistently to manage weeds (Preston and Reiger 2000). Currently, glyphosate-resistant cotton is restricted to a maximum of two 1.5 kg ha^{-1} applications of glyphosate (equivalent to 1035 g ae ha^{-1}) before the 4 true leaf stage and a further 1.5 kg application as a directed or shielded spray (Jones and Snipes 1999).

The experiments described in this chapter were conducted to gain an understanding of how weed management in a glyphosate tolerant cotton system affects the population dynamics of two key grass weeds. The weeds were exposed to a variety of weed management regimes ranging from glyphosate only to a system using all available conventional methods of control. The species monitored were barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) and liverseed grass (*Urochloa panicoides* Beauv.). These two weeds were among the most important weeds found in fallow in northern New South Wales (Felton *et al*. 1994). Walker *et al*. (2005) reported that they were also important weeds in dryland cotton systems. These species also produce large amounts of seed, germinate in high numbers (Keeley and Thullen 1989; Norris 1991; Veenendaal *et al*. 1996) and are easily controlled by glyphosate (Lanie *et al*. 1993; Norris *et al*. 2000), making them candidates for resistance evolution with increasing selection pressure from glyphosate.

4.2 MATERIALS AND METHODS

4.2.1 Experimental site

In all years the experiment was conducted at the Australian Cotton Research Institute at Narrabri, New South Wales. The chosen field was known to have a high grass weed population that would be suitable for the experiment. The soil type in the field was a self-mulching grey clay vertisol (26% sand, 12% silt, and 62% clay).

4.2.2 Experimental design

The experiment was designed as a Latin square with five treatments and five replicates, with a plot size of 40 m x 8 m. Measurements were taken from the inside 20 m x 4 m of each plot, allowing for a buffer between each experimental unit. Each plot consisted of six permanent quadrats (50 cm x 50 cm) from which measurements were taken. These quadrat points were measured from a permanent reference point so that the position of the quadrats could be maintained as close as possible to the original location each season.

The experiment consisted of the following treatments:

1. Roundup Ready herbicide only (RR Only),
2. Roundup Ready herbicide plus a combination of conventional weed management practices (RR + IWM),
3. Roundup Ready herbicide plus a reduced residual herbicide program (RR + Res.),
4. Roundup Ready herbicide plus a grass herbicide (RR + Grass),
5. A combination of conventional weed management practices only (IWM Only).

The conventional weed management practices (RR + IWM and IWM Only) represent the systems already employed in the cotton industry in Australia (Roberts 1998*a*). Such an IWM system includes the use of residual herbicides applied one month prior to planting, at planting and as a lay-by application mid-season. Also included are post-emergent herbicides, cultivation and hand-hoeing (Charles *et al*. 1995). The other three systems are possible systems that could be used with a greater dependence on glyphosate. Since the systems experiment focused on grass weeds, the post-emergent herbicide used was haloxyfop-methyl. An outline of each treatment is given in Appendix 4.1.

4.2.3 Experimental procedure

In all years the field was fertilized with anhydrous ammonia at a rate of 180 kg N ha^{-1}. The field was then cultivated to form beds one meter apart. The field was irrigated one week prior to planting, and then fornightly from six weeks after planting until maturity.

The first soil-applied residual herbicides were applied to their respective treatments approximately one month prior to planting. Those plots were then cultivated to incorporate the herbicides (Table 4.1). The second application of soil-applied residual herbicides occurred two days after cotton planting, glyphosate was also applied at this time. In the third year of the experiment paraquat and diquat (Sprayseed®) were applied to control volunteer glyphosate-resistant cotton plants, just after planting but prior to crop emergence.

In 2003, the cotton variety 'Sicot 289 Bollgard II Roundup Ready' was planted. In the following years, a related variety 'Sicot 71 Bollgard II Roundup Ready' was used. Roundup Ready herbicide was applied as described on the herbicide label, consisting of two 1.5 kg ha^{-1} applications before the fourth true leaf stage of the cotton. The last application of 1.5 kg ha^{-1} Roundup Ready herbicide involved a directed spray just prior to canopy closure. Other herbicide applications and cultivations in their respective treatments were applied as needed.

Table 4.1. Herbicides, herbicide rates and timings used in each treatment in systems experiment in the 2003-04 season. Treatments applied in successive seasons are located in Appendix 4.1

	RR Only		RR + IWM		RR + Res		RR + Grass		IWM Only	
Date	Herbicide	Rate g ai/ha	Herbicide	Rate g ai/ha	Herbicide	Rate g ai/ha	Herbicide	Rate g ai/ha	Herbicide	Rate g ai/ha
4/09/2003			trifluralin	1104					trifluralin	1104
			diuron	1530					diuron	1530
			(cultivation to incorporate trifluralin/diuron)							
17/10/2003	glyphosate[a]	450	glyphosate	450	glyphosate	450	glyphosate	450	glyphosate	450
(Cotton planted)			fluometuron	748	fluometuron	748			fluometuron	748
			prometryn	748	prometryn	748			prometryn	748
			pendimethalin	990	pendimethalin	990			pendimethalin	990
11/11/2003	glyphosate	1035	glyphosate	1035	glyphosate	1035	glyphosate	1035		
26/11/2003	glyphosate	1035	glyphosate	1035	glyphosate	1035	glyphosate	1035		
28/11/2003									haloxyfop	78
15/12/2003	glyphosate	1035	glyphosate	1035	glyphosate	1035	glyphosate	1035		
			prometryn	1080	prometryn	1080			prometryn	1080
27/01/2004							haloxyfop	78		
30/04/2004	(Cotton picked)									

4.2.3.1 Weed populations

Seedling emergence and survival under the various treatments were measured. Measurements on weed numbers were taken at every new weed emergence to record emergence patterns, and the number of survivors of weed control actions throughout the season. Once the cotton reached canopy closure, further germinations all but ceased, so measurements did not need to be taken as regularly.

Plate 4.2 Irrigation of field in systems experiment.

4.2.3.2 Seed bank measurements

The size of the soil seed bank was determined at the start of each season. Sixteen soil cores, 8 cm in diameter and 10 cm deep were taken in each plot, as this was the maximum depth that Dawson and Bruns (1962) found that barnyardgrass seeds would germinate. The soil cores were taken within 1 m either side of the permanent quadrats with an additional two cores taken at specific positions within the plot. Soil cores were then mechanically washed through sieves and weed seeds counted.

4.2.4 Statistical analysis

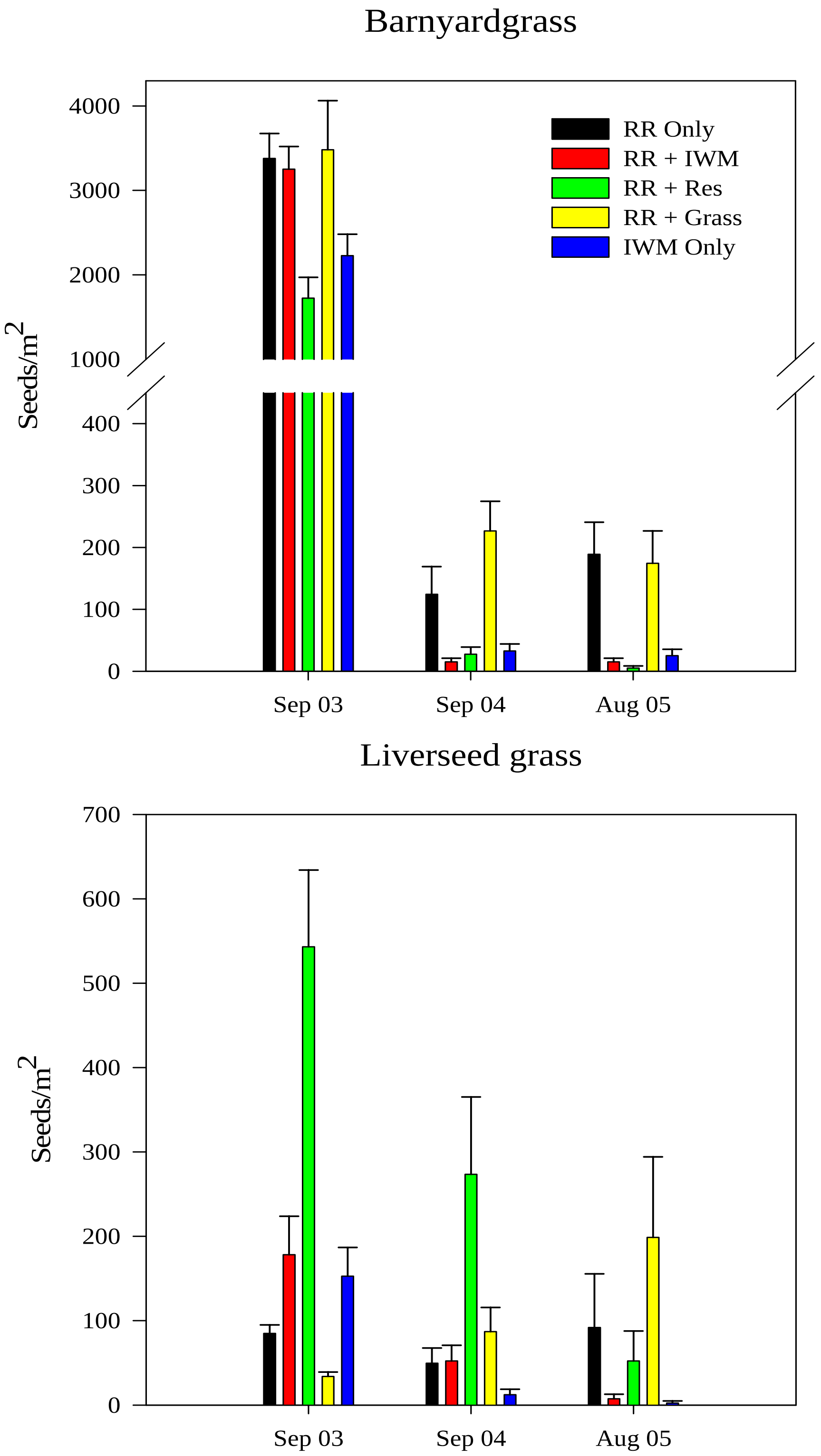
Data on each weed count were analysed by ANOVA (GenStat 7th edn., VSN International, Hertz, UK) to assess differences between weed control treatments applied. This data was transformed by square root, log (base 10) and log (base e), however the transformations did not improve the residuals, therefore data presented is untransformed. Soil seed bank data were also analysed by ANOVA to assess differences between treatments.

4.3 RESULTS

4.3.1 Seed bank measurements

At the beginning of the experiment, all treatments had very high numbers of barnyardgrass seeds in the seed bank (Figure 4.1). The highest soil seed bank was in the RR + Grass treatment with almost 3500 seeds m^{-2}. The RR + Res. treatment had 1700 seeds m^{-2} in the seed bank, while the remaining treatments all had barnyardgrass seed bank densities of over 2000 seeds m^{-2}. This provided a relatively even starting point to measure the effects of weed management practices on subsequent seed bank densities. In September 2004, after the first season of the experiment, barnyardgrass seed bank numbers were reduced considerably in all treatments. For example, the RR + Grass seed bank density declined from 3500 to 230 seeds m^{-2} in September 2004 with the seed bank density in this experiment continuing to decline to 174 seeds m^{-2} in August 2005. The effects of the soil-applied residual herbicides became apparent over time, reducing the number of barnyardgrass in the seed bank more effectively than the treatments that did not receive residual herbicides. The RR + IWM treatment was an example of this, starting with a seed bank of 3250 seeds m^{-2} in September 2003, declining to 15 seeds m^{-2} in September 2004 and August 2005.

Figure 4.1 Soil seed bank densities for barnyardgrass and liverseed grass under the various weed management treatments in the field. Bars indicate standard error of the mean.



The initial liverseed grass seed bank densities were more varied and lower than those of barnyardgrass. As Figure 4.1 illustrates, the RR + Res treatment had an initial density of 540 seeds m^{-2}, whereas other treatments were less than 200 seeds m^{-2}. The higher variation in this treatment was due to a heavier infestation of liverseed grass in one replicate of the treatment. The response of liverseed grass to the treatments was similar to that of barnyardgrass, although not as pronounced, with residual herbicides also proving more effective in reducing the densities of seeds in the seed bank. The density of seeds in the RR + Res. treatment was reduced to just over 50 seeds m^{-2}. Seed bank densities in the RR + IWM and IWM Only treatments declined from 178 and 153 seeds m^{-2} to 7 and 2 seeds m^{-2} respectively. The RR + Grass treatment, in contrast, rose during the experiment from an initial density of 35 seeds m^{-2} to just under 200 seeds m^{-2} by the end of the experiment.

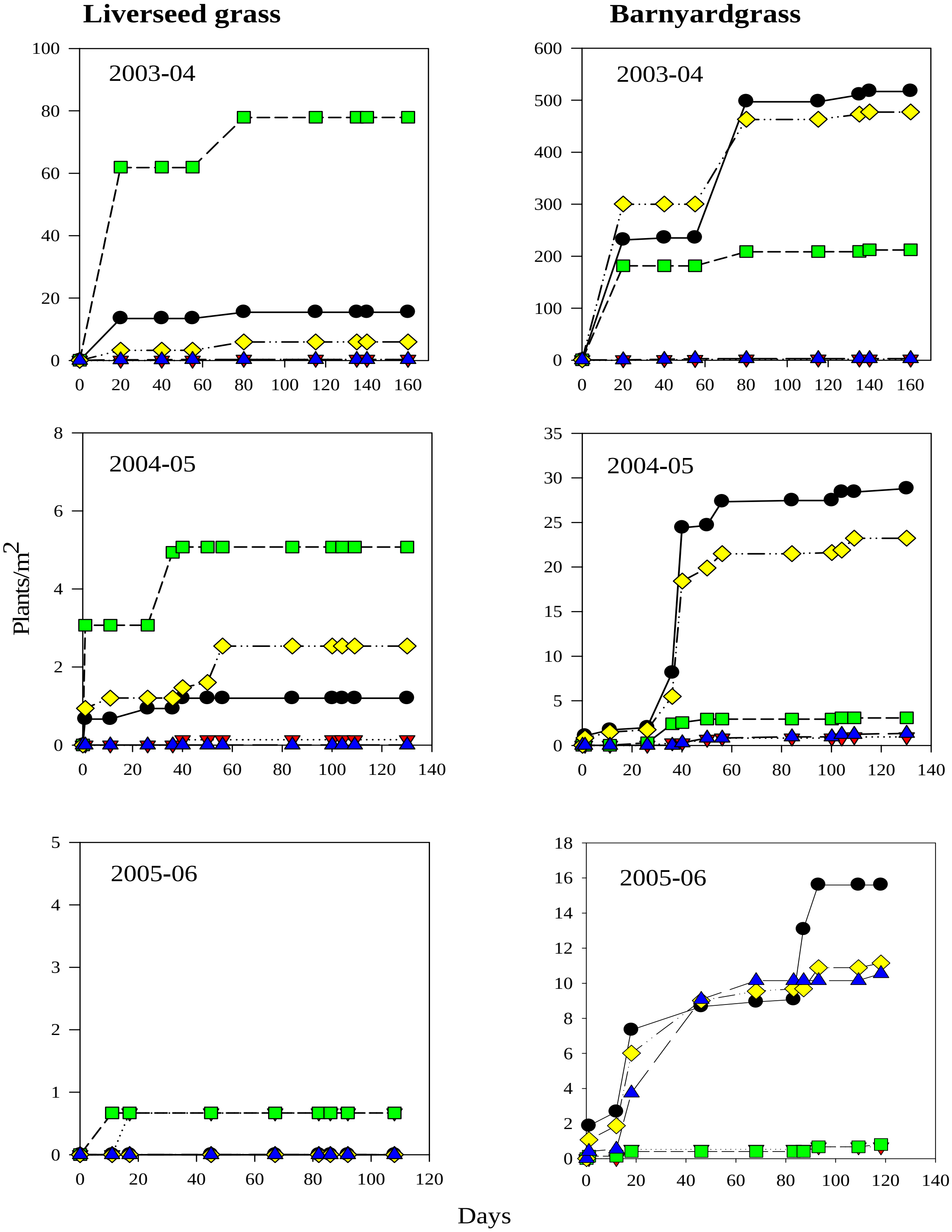
<u>4.3.2 Weed emergence</u>

The weed management regime used had a significant effect on the number of barnyardgrass and liverseed grass plants that germinated (Figure 4.2). For barnyardgrass, this effect was greatly influenced by the application of soil applied residual herbicides. Treatments that received a soil-applied residual herbicide tended to have lower populations of barnyardgrass emerging. The situation was similar for liverseed grass, only the differences were less pronounced. There were large initial germinations of barnyardgrass in the first year of the experiment in the treatments that did not receive the initial residual herbicides before cotton planting (RR Only, RR + Res. and RR + Grass). These treatments had barnyardgrass germinations in excess of 150 plants m^{-2}, with the RR + Grass treatment having 300 plants m^{-2} germinate. In contrast, the RR + IWM and IWM Only treatments had no germinating grasses at the beginning of the experiment. The effect of the residual herbicides was similar for liverseed grass with the RR + IWM and IWM Only treatments having very few germinations, compared to germinations of up to 60 plants m^{-2} in the RR + Res. treatment.

The RR + Res. treatment received a residual herbicide application of fluometuron, prometryn and pendamethalin just after the cotton was planted and this greatly reduced further germination of barnyardgrass. This treatment had only 30 plants m^{-2} germinate for the rest of the season, compared to further germinations of 275 and 176 plants m^{-2} for the RR Only and RR + Grass treatments respectively. The effect of the residual treatment in the RR + Res. treatment was not so pronounced with liverseed grass. Although further germinations were reduced, this treatment still had more additional liverseed grass plants germinate throughout the season than the other treatments. This was most likely due to the greater initial seed bank density of this treatment.

The seed bank studies showed that all treatments were effective in reducing the number of seeds in the seed bank. This in turn led to reduced numbers of weeds germinating in the following seasons of the experiment. The highest number of barnyardgrass plants germinating in later seasons were in the RR Only treatment with less than 30 plants m^{-2} in 2004-05 and 15 plants m^{-2} in 2005-2006. The IWM Only treatment had higher than expected germinations in 2005-06. This occurred because of poor post-emergent control by haloxyfop of residual plants late in 2004-05. The number of liverseed grass plants germinating was also greatly reduced in all treatments following the first season of the experiment, with only 5 plants m^{-2} germinating in the RR + Res. treatment in 2004-05 and less than 1 plant m^{-2} germinating in the RR + Res. and RR + IWM treatments in 2005-06.

Figure 4.2 Total germinations of liverseed grass and barnyard grass in the field from the initial irrigation of the field in preparation for cotton planting (note the variation in scale along the y axis): (●) RR Only, (▼) RR + IWM, (■) RR + Res, (◇) RR + Grass and (▲) IWM Only.



4.3.3 Overall weed numbers

The ability of the residual herbicides to stop germination of barnyardgrass seedlings was reflected in the number of barnyardgrass plants throughout the season. This effect was highly significant, as is shown in Table 4.1. Initial densities of barnyardgrass plants were approximately 200 plants m^{-2} for the RR Only and RR + Res. treatments and almost 300 plants m^{-2} in the RR + Grass treatment. The two IWM treatments had no barnyardgrass plants recorded due to good control by the residual herbicides. The two over-the-top applications of glyphosate provided good control of barnyardgrass plants, reducing densities to less than 10 plants m^{-2} in the RR + Grass treatment, and less than 2 plants m^{-2} in the other treatments.

The effect of the residual herbicides applied at planting and as a lay-by mid-season can be seen in the RR + Res. treatment at the end of November in 2003, with plant numbers in this treatment significantly lower than in the RR Only treatment and much lower than the RR + Grass treatment. From this point on in 2003, the RR + Res. treatment proved to be very effective at controlling barnyardgrass plants, ultimately resulting in the lowest plant density of 0.13 plants m^{-2} at the end of the 2003-04 season.

Table 4.2 Density of barnyardgrass plants throughout the duration of the glyphosate-resistant systems experiment.

Date	RR Only	RR + IWM	RR + Res.	RR + Grass	IWM Only	F. pr	l.s.d (p=0.05)
	Density (plants m^{-2})						
20/10/03	231.07	0.00	180.67	299.47	0.00	0.006	166.90
10/11/03	52.80	0.40	48.40	60.00	0.80	<.001	23.19
21/11/03	1.47	0.13	1.20	9.47	2.13	0.027	5.64
26/11/03	265.87	0.67	28.40	172.27	1.47	0.008	152.8
28/01/04	19.87	0.00	3.47	14.27	0.53	0.018	12.85
29/03/04	4.67	0.67	0.13	3.87	0.40	0.002	2.27
7/09/04	1.07	0.00	0.00	0.80	0.00	0.129	1.08
12/10/04	8.13	0.27	2.40	5.47	0.53	0.166	7.45
15/11/04	19.60	0.27	0.67	17.20	0.40	<.001	6.18
29/11/04	0.67	0.00	0.00	0.13	0.27	0.278	0.71
10/01/05	2.93	0.13	0.13	3.47	0.80	<.001	1.30
31/01/05	0.67	0.13	0.00	1.60	0.40	0.051	1.09
24/03/05	1.73	0.13	0.00	1.07	0.37	0.006	0.89
3/11/05	7.33	0.67	0.27	6.27	3.47	0.088	6.09
23/11/05	0.00	0.00	0.00	0.00	0.00	0.000	0
30/11/05	0.27	0.00	0.00	0.53	1.07	0.445	1.38
8/12/05	0.67	0.00	0.00	0.53	1.07	0.396	1.34
20/12/05	0.27	0.00	0.00	1.07	0.00	0.116	0.93
3/01/06	0.13	0.00	0.00	0.53	0.00	0.062	0.41
30/01/06	3.60	0.13	0.27	1.87	0.00	0.014	2.15
13/03/06	2.93	0.13	0.40	2.13	0.40	0.004	1.48

The effect of residual herbicides proved significant throughout the first season of the experiment. However, the significance of the residual herbicides was somewhat diminished for the rest of the experiment as all treatments greatly reduced the numbers of barnyardgrass plants in the field. Although the RR Only and RR + Grass treatments were unable to stop later-germinating plants due to the lack of residual herbicides, the glyphosate applications provided good control of plants and no further germination occurred after canopy closure. By the end of each season in the experiment, the treatments that received residual herbicides had significantly lower barnyardgrass populations than those that did not.

With respect to liverseed grass, all treatments provided good control (Table 4.2), with no surviving plants at the end of each season. This greatly reduced the numbers of liverseed grass germinating to the point where in 2005-06 only 0.13 plants m^{-2} were recorded for the RR + IWM and RR + Res. treatments, with no liverseed grass plants present in the other treatments.

Table 4.3 Density of liverseed grass plants throughout the duration of the glyphosate-resistant systems experiment.

Date	RR Only	RR + IWM	RR + Res.	RR + Grass	IWM Only	F. pr	l.s.d (p=0.05)
	Density (plants m^{-2})						
20/10/03	13.47	0.00	61.87	3.20	0.13	0.270	66.90
10/11/03	2.80	0.00	2.80	1.07	0.13	0.437	4.21
21/11/03	0.53	0.00	0.93	0.27	0.13	0.660	1.45
26/11/03	2.53	0.27	16.93	2.93	0.00	0.568	24.86
28/01/04	0.00	0.00	0.00	0.00	0.00	-	-
29/03/04	0.00	0.00	0.00	0.00	0.00	-	-
7/09/04	0.67	0.00	3.07	0.93	0.00	0.535	4.28
12/10/04	0.40	0.00	6.53	1.20	0.00	0.472	8.84
15/11/04	0.53	0.13	0.67	0.53	0.00	0.302	1.38
29/11/04	0.00	0.00	0.00	0.00	0.00	-	-
10/01/05	0.00	0.00	0.00	0.00	0.00	-	-
3/11/05	0.00	0.13	0.13	0.00	0.00	0.445	0.23
23/11/05	0.00	0.00	0.00	0.00	0.00	-	-
30/11/05	0.00	0.00	0.00	0.00	0.00	-	-
8/12/05	0.00	0.00	0.00	0.00	0.00	-	-

Similar to barnyardgrass, the effect of residual herbicides on initial densities of liverseed grass plants could be seen, although this was not significant due to the variation in liverseed grass density throughout the field. The effect of the residual herbicides applied at planting and as a lay-by mid season in the RR + Res. treatment was reduced for this reason also. Control of this species with glyphosate applications in the RR + Only treatment and the addition of haloxyfop in the RR + Grass treatment proved to be highly effective. In part, this was a result of liverseed grass tending to germinate early in the season only and well before the post-emergent herbicides were applied, reducing the need for mid-season lay-by residual herbicides.

4.4 DISCUSSION

This systems experiment demonstrated the importance of soil-applied residual herbicides in managing barnyardgrass and, to a lesser extent, liverseed grass populations in a cotton system. However, it also demonstrated that glyphosate applications in the glyphosate-resistant cotton system also provided good control of both weeds. At the end of the first season, all treatments finished with plant densities that were commercially acceptable. These lower plant densities achieved by all treatments greatly reduced the weed numbers in subsequent seasons.

The high rates of glyphosate used in a glyphosate-resistant system provided good control of weeds that germinated within the application window. As will be shown in Chapter 6, and in this experiment with treatments that did not receive residual herbicide application, these high rates were also able to control weeds that germinated early when no residual herbicides had been applied and were older at the time of application. In experiments using glyphosate-resistant cotton in the United States (Culpepper and York 1998), it was reported that soil-applied herbicides generally increased late-season weed control in systems where glyphosate was applied only once. They also reported that soil-applied herbicides did not increase control of any species when glyphosate was applied twice, at 3 to 4 weeks after planting and again at 6 to 7 weeks after planting. These results are supported by this experiment, with overall weed numbers at the end of the season being low in all treatments.

The effect of the residual herbicides was more pronounced in barnyardgrass than liverseed grass. Barnyardgrass is able to germinate almost continually throughout the season (Keeley and Thullen 1989). Although the numbers of plants germinating tended to decrease as the season progressed, the effectiveness of the residual herbicides was still apparent. In contrast, liverseed grass had only two major germinations, with the last germination occurring before November 25 in the first season of the experiment. This reduced the need for the residual herbicides as all these plants could be controlled by post-emergent herbicides. Roberts (1999) reported that pre-emergent residual herbicides only made a small impact on the density of liverseed grass germinations. The experiments conducted by Roberts (1999) were at a non-irrigated site, where the effectiveness of soil-applied residual herbicides is very dependant on incorporation by rainfall and results can vary dramatically depending on the amount of rainfall received. At an irrigated site, such as the one used here, soil-applied residual herbicides were more effective against liverseed grass.

In the IWM Only treatments in the 2003-04 and 2004-05 seasons, haloxyfop was used once each season to control barnyardgrass plants that germinated despite residual herbicides having been applied (Appendix 4.1). Despite this, the numbers of barnyardgrass plants steadily increased throughout the experiment. This resulted in comparatively higher barnyardgrass germinations and created a heavier reliance on haloxyfop to control germinating plants that escaped residual control in the 2005-06 season. This also highlights the benefits of glyphosate use in a glyphosate tolerant system, enabling multiple applications of a post-emergent herbicide with the option of using different herbicide modes of action to successive weed germinations.

At the beginning of the 2005-06 season, the whole experiment was accidentally sprayed with pendimethalin. While this was not a concern for the RR + IWM and IWM Only treatments that were due to receive an application of pendimethalin, it likely had an effect on the other treatments. To combat this, remedial measures of a rotary harrowing to mix the pendimethalin through the profile, an irrigation and delayed planting to minimise the effects of the herbicide were taken. While these measures proved to be quite effective, measurements taken from this season were not used in forming the parameters for the glyphosate resistance model in Chapter 7.

Early season weed control is very important for both barnyardgrass and liverseed grass to reduce weed seed production and thereby minimize long-term weed seed banks (Bosnic and Swanton 1977). All treatments in this experiment contained measures for early season weed control whether it was in the form of soil-applied residual herbicides, glyphosate or haloxyfop as post-emergent controls or both. The result was an overall reduction in the seed bank for both species. However, control of later germinations and escapes with the current glyphosate-resistant system when reliance is shifted to glyphosate alone is a matter of increasing importance in terms of seed bank and resistance management. Glyphosate used in conjunction with residual herbicides has been shown to provide excellent weed control and high crop yields (Isgett *et al*. 1997; Keeton and Murdock 1997). The current experiment was conducted on species that were easily controlled by glyphosate and the benefits from the use of residual herbicides would be more evident on species that are harder to control with glyphosate (Culpepper and York 1998). The lack of residual activity of glyphosate means that it will often need to be applied more than once to control weeds that have extended germination such as barnyardgrass did in this experiment. The residual herbicides help control these weeds and allow cotton to form a virtually weed-free canopy that lasts until harvest (Culpepper and York 1998; Grichar *et al*. 2004).

The RR + Res. treatment provided the more favourable weed control option, as overall weed control was as good as both the IWM treatments, with less weed management inputs involved. While this management strategy appears effective, it is important to gain an understanding of the long-term impacts of each of the treatments on glyphosate resistance evolution. The model in Chapter 7 will provide an understanding of resistance evolution and enable the determination of preferred weed management strategies for both weed control and resistance prevention.

CHAPTER 5

ABOVE-GROUND BIOMASS AND SEED PRODUCTION OF BARNYARDGRASS AND LIVERSEED GRASS IN COMPETITION WITH COTTON.

5.1 INTRODUCTION

Barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) is one of the most important annual weeds in summer-growing crops, and has been reported to be a problem in 36 different crops in at least 61 countries (Holm *et al*. 1977; Maun and Barrett 1986; Norris *et al*. 2001). Liverseed grass (*Urochloa panicoides* Beauv.) is also an important weed in northern Australia, with liverseed grass and barnyardgrass being listed among the most important weeds found in fallows in northern NSW (Felton *et al*. 1994). They are also important weeds in dryland cotton systems (Walker *et al*. 2005). The growth of liverseed grass in summer can be a major problem as it can rapidly deplete soil moisture and nutrients (Adkins *et al*. 1998). Both species are highly competitive with crops, and Roberts (1999) reported that, uncontrolled liverseed grass reduced yield by up to 61 percent. Barnyardgrass can also have detrimental effects on cotton yield (Keeley and Thullen 1991).

Barnyardgrass has early, rapid, and continual emergence (Keeley and Thullen 1989), it can produce seed over a long period if conditions permit. Even at densities of less than 1 plant m^{-1} of row, Norris (1991) reported that barnyardgrass plants are able to produce an average of 100,000 seeds $plant^{-1}$. In competition with cotton in the United States, barnyardgrass plants produced 350 seeds $plant^{-1}$ at 9 weeks (Keeley and Thullen 1991), this increased to 4,400 seeds $plant^{-1}$ at 12 weeks. Seed production per plant decreases as plant density increases, and later emerging plants produce less seed (Bosnic and Swanton 1997) as decreasing day lengths hasten flowering (Keeley and Thullen 1989).

Veenendaal *et al*. (1996) found that liverseed grass seed production ranges from 200 to 2,400 seeds m^{-1} of row in the savannas in Botswana. To date, seed production and biomass of liverseed grass in irrigated cotton production systems have not been recorded, and it is expected that biomass and seed production in these systems would be higher due to the more favourable conditions.

The purpose of this study was to determine the seed production and biomass of barnyardgrass and liverseed grass when grown in competition with cotton under a range of weed planting densities. Although there is considerable information on the seed-producing capabilities of barnyard grass in a number of crops outside Australia, there is no information on its characteristics when grown in irrigated cotton systems under Australian conditions. Results from this experiment will also provide information on seed production that will be used in the construction of a population dynamics model in Chapter 7 to determine the potential of the two species to develop glyphosate resistance under a range of management practices.

5.2 MATERIALS AND METHODS

5.2.1 Experimental sites

The experiments were conducted at the Australian Cotton Research Institute at Narrabri, New South Wales ($30^o13'$S, $149^o47'$E) during the 2003-04 and 2004-05 seasons (temperature and rainfall graph is located in Appendix 5.1). In the first year, the experiment was conducted in fibro boxes, called polycages, situated outside. The polycages were set out in rows of eight, with each individual polycage representing an experimental unit having dimensions 1 m (l) x 1 m (w) x 50 cm (d) (Plate 5.1). The experiment was set up with water and nutrients not limited, so the full potential of barnyardgrass and liverseed grass biomass and seed production could be achieved. In the second year, the experiment was conducted in a cotton field under normal irrigated cotton growing conditions so that the characteristics of these weeds could be better understood (Plate 5.2).

Plate 5.1. Polycages where the experiment examining the growth and seed production of barnyardgrass and liverseed grass was conducted in 2003-04.

Plate 5.2. Field where the experiment examining the growth and seed production of barnyardgrass and liverseed grass was conducted in 2004-05 (barnyardgrass plants are the taller plants in the middle of the picture, with liverseed grass plants to the right).

5.2.2 Experimental Design

In the 2003-04 season, the experiment conducted in polycages was a completely randomised design with four replications. Barnyardgrass and liverseed grass were planted at densities of 0, 5, 10, 20, and 50 plants m^{-1} of row. The cotton, variety CSX519 (Sicot 289 Bollgard II Roundup Ready®), was planted at a density of 12 plants m^{-1} of row. This is similar to the planting density used in commercial cotton production.

The field experiment in the 2004-05 season was a randomised block design with species and planting date blocked for practicality of planting. The experiment contained both weed species with three planting dates and four replications. The first planting date coincided with cotton planting in the field; the second after the fourth true leaf stage of the cotton; and the third mid-season planting was about 10 days prior to full canopy closure of the cotton crop. The last two plantings were timed to occur after the final over-the-top and directed Roundup Ready® sprays, respectively, to obtain an understanding of seed production and biomass of cohorts that germinate in the field after these events. Densities used in the experiment were 0, 0.5, 1, 2, 5, 10, and 50 plants m^{-1} of row. These lower densities were chosen to be typical of economic threshold levels and provide useful information for making long-term decisions rather than estimating enormous seed rain from high densities of uncontrolled weeds (Norris *et al*. 2001; Norris 2004). The size of each plot was 4 m x 3 m rows with measurements coming from the middle row. Other weeds that emerged throughout the trial were controlled by hand.

Plate 5.3. Emerging liverseed grass seedlings that were planted at the second planting date after the fourth leaf stage of the cotton crop.

5.2.3 Experimental procedure

In 2003-04 the soil, (self-mulching grey vertisol; 26% sand, 12% silt, 62% clay) in the polycages was taken from the research station. The polycages were fertilised with 100 kg/ha of slow release fertiliser with analysis of 15:4:7 N:P:K plus trace elements prior to cotton planting. Throughout the experiment each box was fertilised with liquid fertiliser with analysis of 24:4:19 N:P:K plus trace elements every two weeks. Cotton was planted into the polycages at 15 plants m^{-1} of row and thinned to 12 plants m^{-1} of row after germination. Weed seeds were planted in the glasshouse the next day. Once the grass seedlings had reached the two-leaf stage, they were transplanted into the polycages at the previously specified densities on both sides of the cotton rows at a distance of 10 cm from the row. At this time the cotton was at the one to two-leaf stage.

The timing of first inflorescences for the weeds was recorded. As the plants produced more mature seed, the seed was collected up to twice weekly and then once weekly for barnyardgrass so that a minimal amount of seed was lost. The weight of 1000 barnyardgrass seeds was determined for each plot so that seed numbers could be estimated from total seed weight. Seed from liverseed grass was initially collected in this way, however due to almost constant seed rain, it was impossible to prevent large seed losses. As liverseed grass has seed heads on which the number of seeds can be counted relatively easily, an alternative strategy for measuring seed production was carried out. This involved taking five inflorescences per replicate just prior to maturity and counting the number of seeds per head. Once plants had matured, four plants per plot were harvested and their above-ground biomass and number of inflorescences were recorded. Seed production was estimated by multiplying the average number of seeds inflorescence^{-1} by the average number of inflorescences plant^{-1}.

In 2004-05 the field was fertilised with 180 kg N ha^{-1} in the form of anhydrous ammonia. Cotton was planted at a rate of 12 seeds m^{-1} of row using a commercial planter. The following day, seeds of the two weed species were planted with a cone planter at a rate assuming 15 percent germination. Seeds were planted offset 10 cm to the left of the cotton row. After germination, the weeds were thinned to the required densities. The germination of barnyardgrass was below that required, so barnyardgrass seeds were immediately planted in the glasshouse and transplanted to the field once they had reached the two-leaf stage. However, this resulted in the highest density achieved in the field for the first planting was 15 plants m^{-1} of row, rather than the desired 50 plants m^{-1} of row.

Once the cotton had reached the four-true-leaf stage, six weeks after the cotton was planted, the second planting of weed seeds was performed as described above, except the barnyardgrass seed was planted assuming a 10 percent germination. Once the weeds had germinated, they were thinned to the required densities. The third planting was conducted mid-season, about 12 weeks after the cotton was planted, and was done by hand due to the size of the cotton.

Seed was collected from the weeds weekly as described above. When the weeds had reached maturity, four plants per plot were harvested and above-ground biomass and inflorescence number were recorded. In the case of liverseed grass, 20 seed heads per plot were collected and the seeds per head counted.

5.2.4 Statistical analysis

Data were analysed by ANOVA (GenStat 7th edn., VSN International, Hertz, UK) to assess whether seed production and biomass of the two species differed between densities. Seed production and biomass of the two species were then fitted to a rectangular hyperbolic model $y=ax/(b+x)$ on a per meter of row basis, and a hyperbolic decay model $y=ab/(b+x)$ on a per plant basis, where a is the maximum seed production per unit biomass, x is the weed density, and b is the change in seed production per unit biomass.

5.3 RESULTS

5.3.1 Vegetative growth

5.3.1.1 Barnyardgrass

In all situations, the impact of density on above ground biomass was significant (Appendix 5.1) as was planting time in 2004-05. Barnyardgrass growth in the field was slightly larger than in the polycages in 2003-04 (Figure 5.1). A possible reason for this is that the plants were confined to the dimensions of the polycages in 2003-04 despite abundance of water and nutrients. Later planting of barnyardgrass reduced final biomass significantly (Figure 5.1). As barnyardgrass density increased, above ground biomass per unit area also increased (data not shown) and biomass per plant decreased (Figure 5.1). At lower densities in 2004-05 average biomass of individual plants was 791 g and 811 g for densities of 0.5 and 1 plants m^{-1} respectively. The average biomass of individual plants at the highest density of 15 plants m^{-1} of row was 128 g. Average biomass of individual plants in the polycages in 2003-04 ranged from 219 g at five plants m^{-1} down to 26 g at 50 plants m^{-1}.

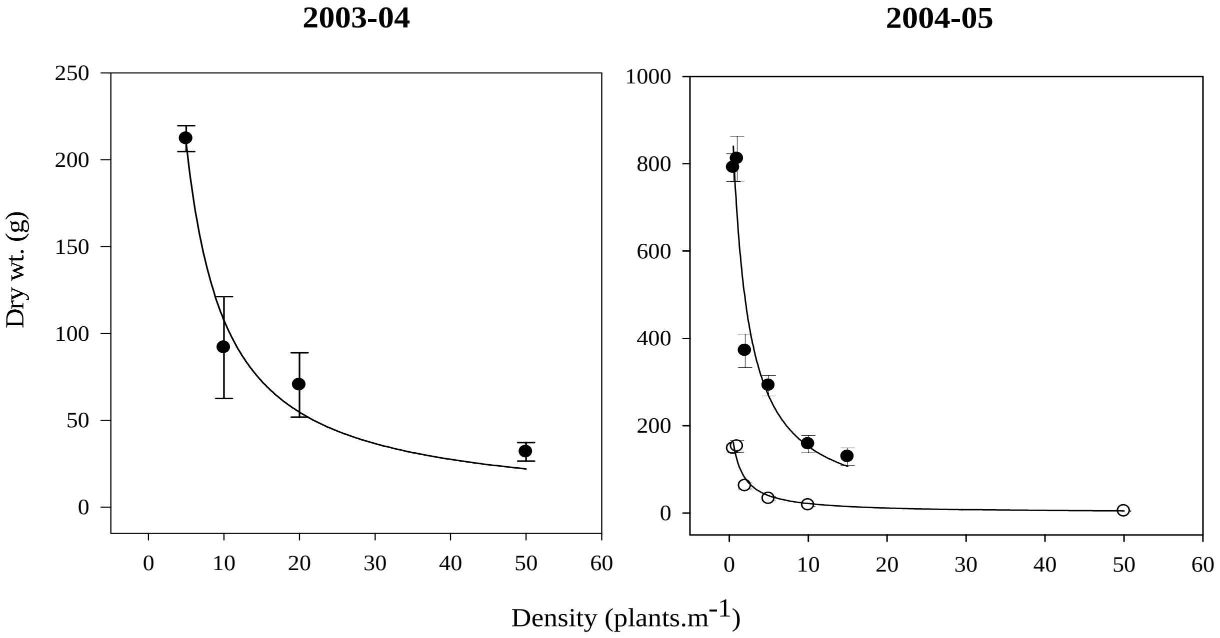


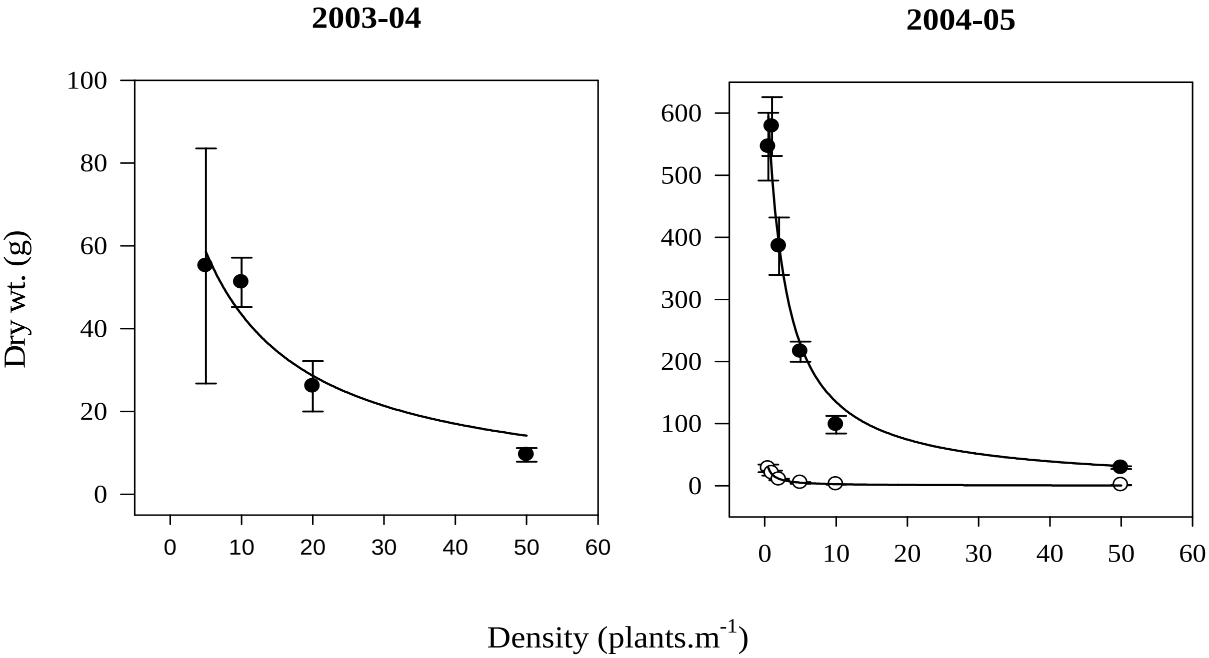
Figure 5.1. Barnyardgrass above-ground biomass per plant. In the 2004-05 graph the first planting is denoted by ●, and the second planting by ○. Regression parameters are listed in appendix 5.4. Bars indicate standard error of the mean.

In 2004-05, barnyardgrass that was planted after the four leaf stage of the cotton crop was significantly smaller than that planted at the same time as the cotton. The average biomass for individual plants ranged from 147 g at 0.5 plants m^{-1} to 4.3 g at 50 plants m^{-1}. This decline in biomass highlights the competitiveness of the cotton crop for water and nutrients once it gets established, along with the effect of shading of the barnyardgrass by the cotton (Maun and Barrett 1986). Barnyardgrass planted just prior to canopy closure of the cotton failed to emerge.

5.3.1.2 Liverseed grass

The growth of liverseed grass was considerably larger in the field in 2004-05 than in the polycages (Figure 5.2). At similar planting densities of 5, 10, or 50 plants m^{-1} for liverseed grass planted at the same time as cotton, average biomass of individual plants was 386, 216 and 98 g, respectively, in the field compared to 55, 51 and 9 g, respectively, in the polycages in 2003-04. In general, as weed density increased, the above-ground biomass per plant decreased and the biomass per meter of row increased. This effect was highly significant in the field for both planting times, but not so in the polycages (Appendix 5.2). This may also indicate the ability of lower densities of liverseed grass to take advantage of the relatively higher amount of nutrients available compared to higher densities in the polycages and also that growth of the higher densities may have been impeded by the cages.

Figure 5.2. Liverseed grass above-ground biomass per plant. In the 2004-05 graph the first planting is denoted by ●, and the second planting by ○. Regression parameters are listed in appendix 5.4. Bars indicate standard error of the mean.



Growth of liverseed grass in the field in 2004-05 was inhibited by the presence of older, more competitive cotton. Biomass of liverseed grass planted after the four leaf stage of the crop ranged from 28 g at 0.5 plants m^{-1} to 1g at 50 plants m^{-1} for individual plants. As seen with barnyardgrass, this is further proof of the competitive ability of cotton once it is established. Like barnyardgrass, the third planting of liverseed grass just prior to canopy closure of the cotton failed to emerge.

5.3.2 Seed production

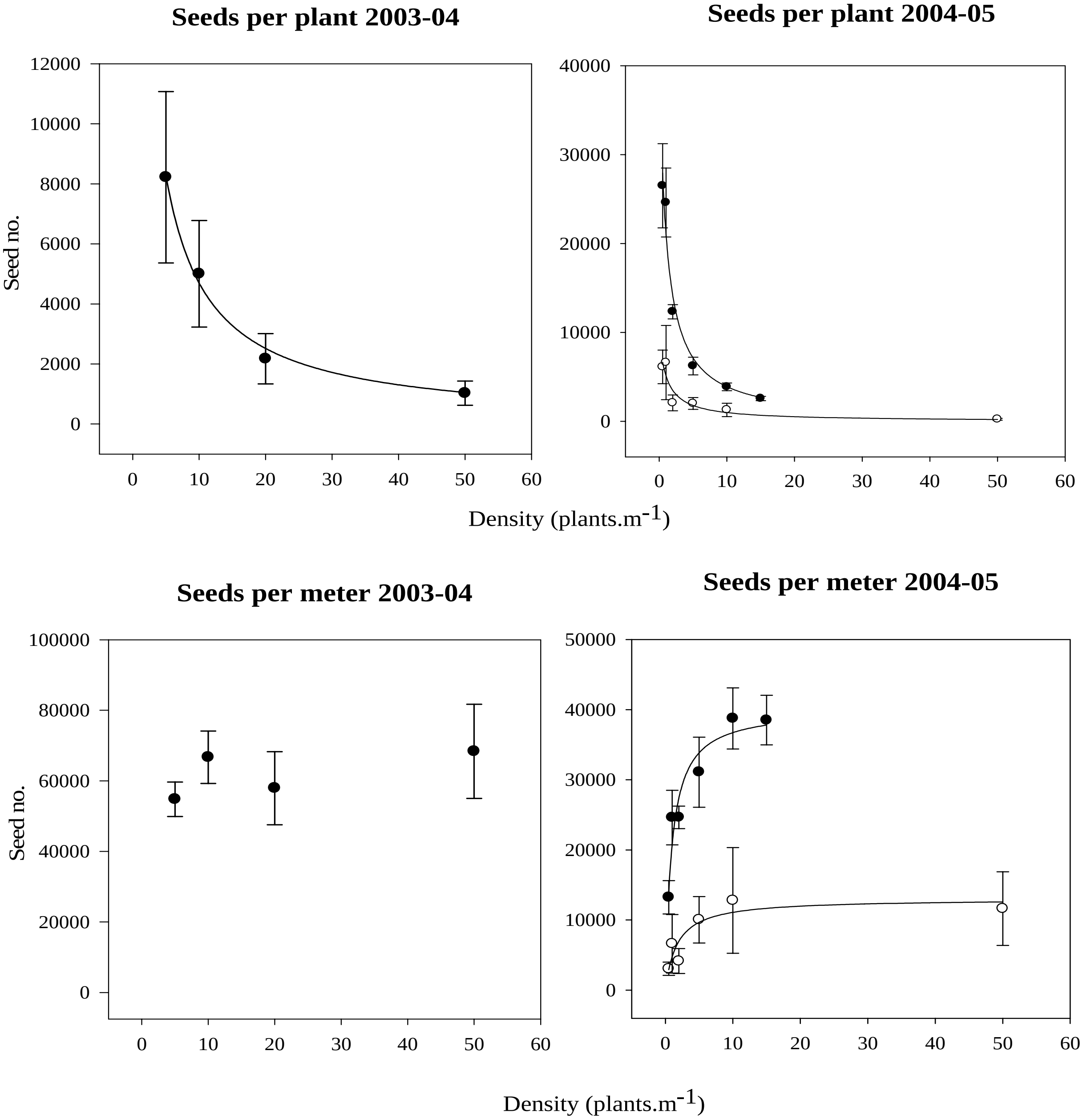
5.3.2.1 Barnyardgrass

Phenological development of barnyardgrass planted at the same time as cotton was not affected by planting density in either experiment. The polycage experiment and field experiment were planted on October 9, 2003 and October 2, 2004, respectively, and initiation of inflorescences was just over 1 week slower in 2004 than 2003. In both experiments, barnyardgrass continued to produce seed throughout the season until late January, with more inflorescences arising from later-growing tillers. In the field experiment in 2004-05, the later planted barnyardgrass was planted on November 25 and germinated on December 6. Days to flowering were the same as for the earlier planting (data not shown).

For barnyardgrass, as density increased, seed production per plant decreased, but seed production per meter of row increased (Figure 5.3). This effect was highly significant for the first planting time in the field in 2004-05, however not so for the second planting time (Appendices 5.2 and 5.3). However, in the polycages differences in seed production per plant were significant, but seed production per meter of row was not. Seed production in the field ranged from an estimated 26,500 seeds plant^{-1} at 0.5 plants m^{-1} of row down to 2500 seeds plant^{-1} at 15 plants m^{-1} of row. This corresponded to nearly 14,000 seeds m^{-1} of row at 0.5 plants m^{-1} of row to almost 40,000 seeds m^{-1} of row at the highest density. In contrast to biomass measurements, the seed production of barnyardgrass was considerably higher in 2003-04 in the polycages than in 2004-05 in the field. Seed production per plant ranged from an estimated 8200 seeds plant^{-1} at the lowest density of 5 plants m^{-1} of row to 1000 seeds plant^{-1} at 50 plants m^{-1} of row. This equated to a total seed production of over 50,000 seeds m^{-1} of row for both densities.

Like biomass, seed production was reduced by between 75 and 90% when barnyardgrass was planted after the four leaf stage of cotton due to shading and competition from the cotton crop. Plants produced 6000 seeds plant^{-1} at 0.5 plants m^{-1} of row, down to 230 seeds plant^{-1} at 50 plants m^{-1} of row. The resulting seed production reached a maximum of just over 12,000 m^{-1}. Bosnic and Swanton (1997) also reported reductions in seed production with older maize. Barnyardgrass seeds emerging up to the three leaf stage produced from 14,400 to 34,600 seeds m^{-2} as opposed to 1200 to 2800 seeds m^{-2} at the four leaf maize stage.

Figure 5.3. Barnyardgrass seed production. In the 2004-05 graph the first planting is denoted by ●, and the second planting by ○. Regression parameters are listed in appendix 5.4. Bars indicate standard error of the mean.



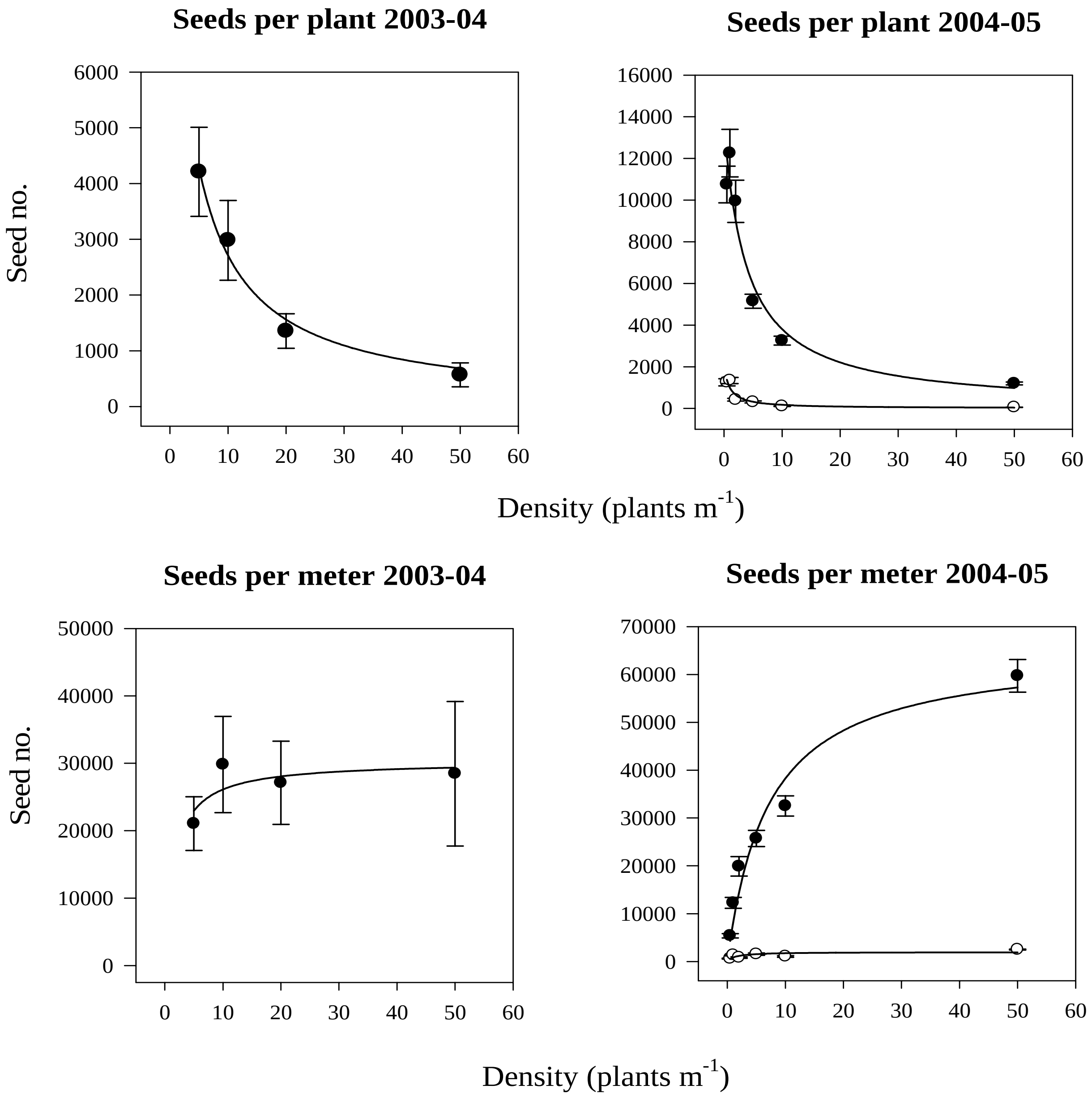
5.3.2.2 Liverseed grass

Planting density did not effect the phenological development of liverseed grass. The time to initiation of flowering differed by just over a week between all liverseed grass germinations in 2003-04 and 2004-05. In general, liverseed grass had slightly faster and more uniform germination than barnyardgrass.

As liverseed grass density increased, seed production plant^{-1} decreased and seed production m^{-1} of row increased (Figure 5.4). This was highly significant in the field in 2004-05 (Appendices 5.2 and 5.3), but seed production m^{-1} of row did not differ significantly with plant density in 2003-04. Again this could be a result of the capacity of liverseed grass to thrive at low densities while also showing evidence for intra-specific competition at higher densities in a confined space. In the polycages, seed production ranged from 4200 seeds plant^{-1} at five plants m^{-1} of row to under 600 seeds plant^{-1} at 50 plants m^{-1} of row. In contrast, liverseed grass in the field produced nearly 11,000 seeds plant^{-1} at 0.5 plants m^{-1} of row down to 1100 seeds plant^{-1} at 50 plants m^{-1} of row. This equated to a maximum of almost 60,000 seeds produced m^{-1} of row in the field compared to half that in the polycages. The increase in seed production in the field reflects the larger plants having a larger biomass with more tillers and, therefore, more inflorescences.

The presence of established cotton also affected seed production of liverseed grass, in this case reducing seed production by 90% with the later planting of liverseed grass in the field. For the later planting of liverseed grass, average seed production ranged from 1250 seeds plant^{-1} at 0.5 plants m^{-1} of row down to less than 100 seeds plant^{-1} at higher densities. This resulted in a maximum seed production of almost 2500 seeds m^{-1} of row. The seed production in all experiments conducted in this study was greater than that reported by Veenendaal *et al*. (1996), reflecting the more favourable conditions in an irrigated cotton field compared to a savanna.

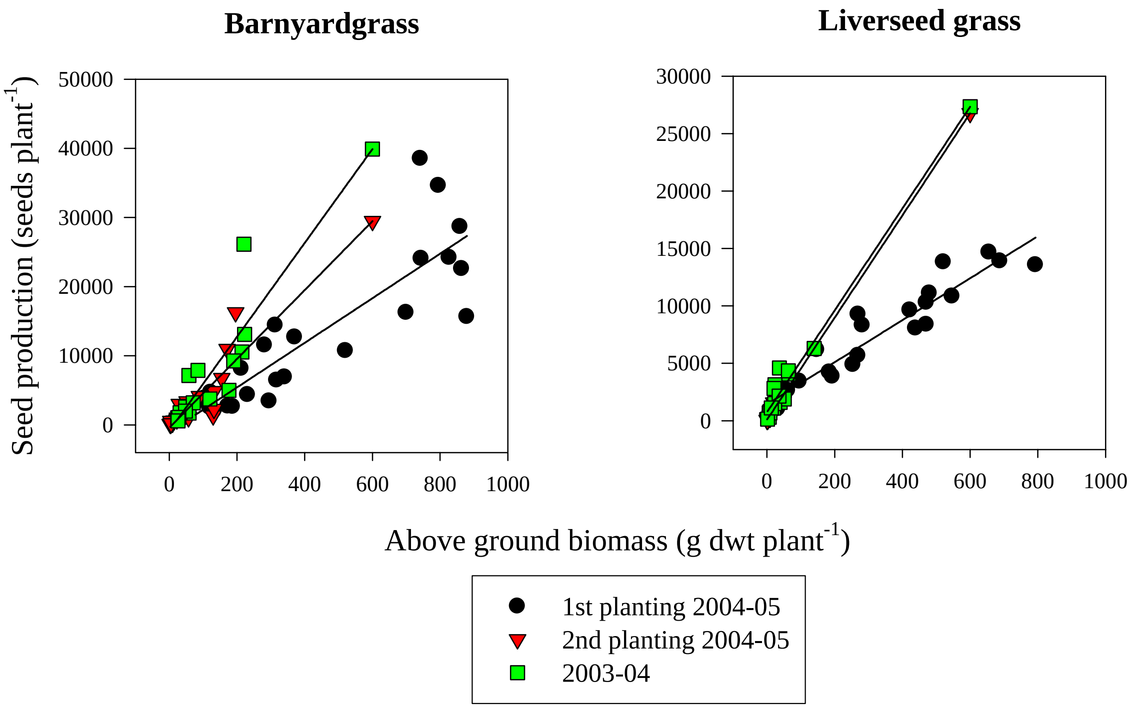
Figure 5.4. Liverseed grass seed production. In the 2004-05 graph the first planting is denoted by●, and the second planting by ○. Regression parameters are listed in appendix 5.4. Bars indicate standard error of the mean.



5.3.3 Reproductive effort

The seed production of barnyardgrass and liverseed grass in relation to above-ground biomass is shown in Figure 5.5 as a measure of their reproductive effort. Both barnyardgrass and liverseed grass showed a liner relationship between biomass and seed production. Reproductive effort for both species was higher in the polycages in 2003-04 than in the field in 2004-05. Weeds planted after the four leaf stage of cotton also had a higher reproductive effort than those planted at the same time as cotton.

Figure 5.5. Seed production per plant expressed in relation to above ground biomass per plant. Regression parameters are listed in appendix 5.4.



Barnyardgrass

Liverseed grass

5.4 DISCUSSION

This study has shown the effects that weed density can have on above-ground biomass and seed production for both species. Above-ground biomass measurements provide a useful indication of how weed growth will affect nutrient uptake and competitiveness with the crop in which they are present. As the experiments were set up with ideal conditions, weeds of both species tended to be larger than expected under normal growing conditions where these weeds would receive herbicide treatments. Barnyardgrass plants, particularly at the higher densities, grew to almost 2 m tall, reflecting their large biomass. However, this experiment illustrated the potential growth of these two weed species if adequate control is not obtained.

When results for barnyardgrass growth from the polycages were compared to those in the field, it did appear that the restraints of the polycages had an effect on above-ground biomass. It is likely that barnyard grass plants extract nutrients from a relatively large area. Although the plants did not appear to be under any stress, it is evident from the seed production and reproductive effort that in the polycages more effort was put into reproduction than for plants growing in the field. The reproductive effort of the weeds planted later in the field was also greater than those planted earlier. This suggests these weeds will produce seed rather than biomass under less favourable growing conditions. In contrast, liverseed grass growth did not appear to be hindered in the polycages.

The timing of weed germination within the cotton crop had a profound effect on weed size and seed production. Weed seed planted just prior to canopy closure failed to germinate. Previously, Keeley and Thullen (1991) reported that a weed-free period of 9 weeks would prevent barnyardgrass seed production, a conclusion supported by this study. Keeley and Thullen (1991) also found that while cotton was not a good competitor with barnyardgrass early in the season, it became a successful competitor within 6 to 9 weeks from cotton emergence. They also reported that barnyardgrass plants emerging 9 or more weeks after cotton died before harvest as they succumbed to shading. In the present study, seeds that were planted just prior to canopy closure failed to emerge, possibly due to absence of light penetration through the canopy of the cotton crop. Bosnic and Swanton (1997) reported similar effects of an established crop of corn on weed seed production. This emphasizes the importance of early weed control in cotton and raises the question as to whether weeds that are present after cotton canopy closure are the result of further germinations or escapes from earlier weed control actions.

The effect of the cotton canopy was also seen on liverseed grass, which had reduced growth and seed production of the second planting and failure to emerge for the third planting. Veenendaal *et al*. (1995) stated that the survival of liverseed grass appears to be enhanced by shading; however, in this experiment, the competitiveness of the cotton crop for light, nutrients and water significantly reduced liverseed growth.

The reproductive output of barnyard grass varies considerably. An individual plant can produce more than 400,000 seeds (Norris 2001). Maun and Barrett (1986) stated that reasons for this could be attributed to several factors, including differential plasticity among biotypes and environmental conditions. The fact that both barnyardgrass and liverseed grass were able to produce large amounts of seed at low weed densities raises an issue with the application of economic thresholds for these two species. Economic thresholds that allow small numbers of these weeds to survive and produce seed will result in a ready replenishment of the weed seed bank (Norris 2001). Where weed control has been poor, that replenishment could be considerable and may result in long-term weed problems. There are also implications for herbicide resistance evolution in these species. If a single surviving resistant plant is able to produce large numbers of seed, the rate of resistance development could increase considerably. In addition, in the case of cotton, later germinations that emerge after the herbicide has been applied will contribute little to dilute the number of resistant plants in the population. Diggle *et al*. (2003) concluded that minimising the weed population size substantially decreases the risk of herbicide resistance evolution, stating that as the population of the weed decreased, so does the chance that a rare resistance gene will be present.

Both species in this study were able to produce a large amount of seed if not controlled early in the season. Therefore, weed management programs for these species must be tailored to prevent early germinating weeds surviving and producing seed. Crop agronomy has also been shown to be important in the management of these weed species. Crops that are managed for good early season growth can also provide useful weed management tools later in the season. Chapter 4 illustrated the staggered germination of barnyardgrass into a number of cohorts throughout the season. Liverseed grass tends to germinate in fewer cohorts, making it perhaps easier to manage in cotton. In this experiment, plants germinating after the four leaf stage of cotton, which corresponds to after the last over-the-top Roundup Ready® spray, were still able to produce a considerable amount of seed. Even with current glyphosate-resistant technology, herbicide applications and inter-row cultivation are unable to provide effective control of weeds in the plant line past the four-leaf stage in the crop. This makes it important that an integrated strategy of weed management involving effective early season herbicide use and crop agronomy be employed.

CHAPTER 6

DOSE-MORTALITY RESPONSE OF BARNYARDGRASS AND LIVERSEED GRASS TO GLYPHOSATE

6.1 INTRODUCTION

Glyphosate (*N*-phosphonomethyl glycine) is an important global herbicide due to its ability to control a broad spectrum of weeds and its relatively low cost of use (Baylis 2000). The effectiveness of glyphosate is largely determined by factors such as application rate and timing (Jordan *et al*. 1997). Efficacy is often influenced by environmental effects on herbicide absorption, translocation and metabolism within the plant (Wyrill and Burnside 1976). However, intrinsic biological factors also play a part (Malik *et al*. 1989; Kirkwood *et al*. 2000; Pline *et al*. 2001). In glyphosate-resistant cotton, over-the-top applications of glyphosate are restricted to the four-leaf developmental stage of the crop (Jones and Snipes 1999).

The recommended rate of glyphosate in glyphosate-resistant in Australia cotton is three applications of 1.5 kg "formulated product" ha^{-1}, two over-the-top and one shielded (Monsanto 2000). After the four-leaf stage, glyphosate must be applied as a directed or shielded spray so there is no foliar contact with the cotton plant (Monsanto 2000). This is due to potential damage to reproductive parts of the plant where a reduction in pollen fertility and consequently yield loss can result (Jones and Snipes 1999; Pline *et al*. 2002). The formulation of Roundup Ready® herbicide is 690 g ae kg^{-1} of glyphosate present as a mono-ammonium salt, so the recommended rate of formulated product equates to a rate of 1035 g ae ha^{-1} glyphosate (1.5 kg ha^{-1} x 690 g ae kg^{-1}) applied each time in the field. The rates used in this experiment were in proportion to the recommended rate. Weed size was also chosen similar to that experienced in the field in relation to the restrictions on glyphosate use in a glyphosate tolerant cotton system.

This experiment was undertaken to determine the levels of control on barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) and liverseed grass (*Urochloa panicoides* Beauv.) attained when using glyphosate in a glyphosate-resistant cotton growing system. Data from this experiment will be applied in the glyphosate resistance model in Chapter 7.

6.2 MATERIALS AND METHODS

6.2.1 Experimental Sites

Experiments were conducted in the field rather than the glasshouse in order gain a better understanding of the weed's response to glyphosate applications in an irrigated Roundup Ready® cotton system. Sites were selected that had a dense and relatively even distribution of weeds for optimal results. In 2003, a suitable site was selected at Cotton Seed Distributors research farm 30km west of Narrabri on a field that had a barnyardgrass and liverseed grass problem the previous year. The site had an average density of 1200 barnyardgrass plants m^{-2}, and 225 liverseed plants m^{-2}. The 2004 experiment was conducted at the Australian Cotton Research Institute (ACRI) in a field that also had a high population of the two grass weeds at densities of 250 barnyardgrass plants m^{-2}, and 50 liverseed grass plants m^{-2}.

In 2005, a suitable site for conducting the experiment could not be found. Therefore, barnyardgrass and liverseed grass seeds were mixed together and then planted with a cone planter in the field to densities of 90 barnyardgrass plants m^{-2}, and 50 liverseed grass plants m^{-2}, each row of weeds offset 10 cm to the left of the cotton row. This enabled the experiment to be conducted at ACRI.

6.2.2 Experimental Design

The herbicide used was Roundup Ready® herbicide. All experiments had a completely randomized design. In 2003, the treatments were 0, 345, 690 and 1035 g ha^{-1} of glyphosate with four replicates. An additional treatment of 172.5 g ha^{-1} of glyphosate was included in the four replicates in 2004 to obtain additional information for dose response curves. In 2005, rates of 0, 86.25, 172.5, 345, 690 and 1035 g ha^{-1} were used with only 3 replicates due to field size restrictions. Glyphosate applications were at 2, 4, and 6 leaf growth stages for the grass weeds in 2003. In the following years, glyphosate applications were at the 2 to 4 and 6 to 8-leaf stages.

Plot sizes for all experiments were 4 m (l) x 2 m (w). Each plot contained three quadrats in which weed numbers were recorded before spraying. The plots were then sprayed with a hand boom with Albuz API 110 015 flat fan nozzles. A spray pressure of 2 bar was obtained using CO_2 as the propellent. The spray output was 100 L ha^{-1} at a speed of 1 m s^{-1}. The numbers of survivors were counted 20 days after treatment to obtain percentage survival information.

6.2.3 Statistical Analysis

Data were analysed by ANOVA (GenStat 7th edn., VSN International, Hertz, UK) to assess the effects of dose rate and growth stage on barnyardgrass and liverseed grass survival. The percent survivors were then fitted to an exponential decay model $y = a*exp(-b*x)$ where a is the maximum survival of weeds without glyphosate application, b is the coefficient of reduction in survival under glyphosate application, and x is the glyphosate dose rate in g ha^{-1}.

6.3 RESULTS

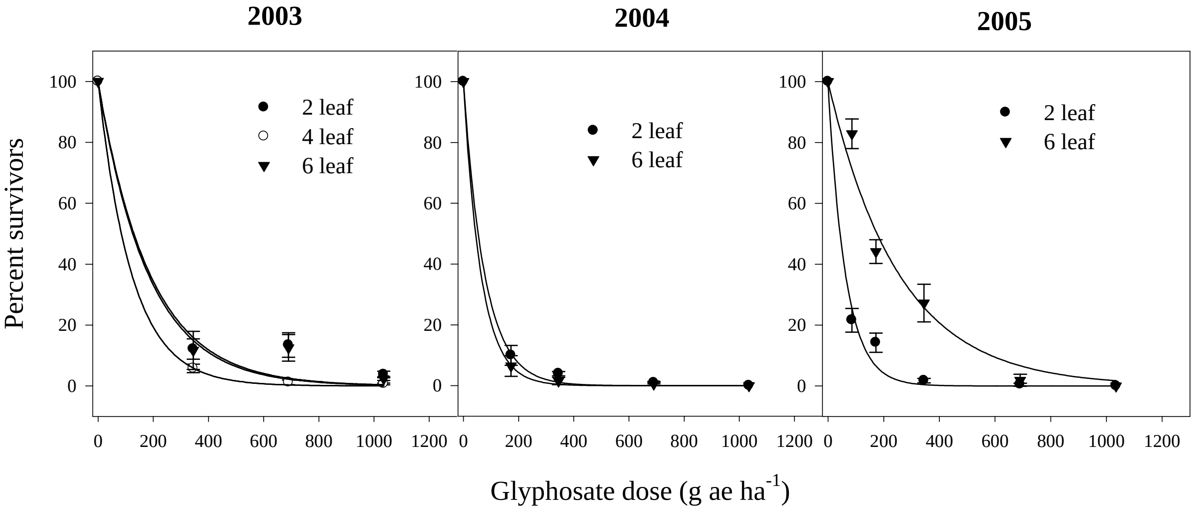
In general, glyphosate provided good control of both barnyardgrass and liverseed grass plants at all growth stages sprayed. As expected as glyphosate dose increased the percent of survivors decreased for both species. In 2003, there was no difference in the response to glyphosate application between the two species (Appendix 6.1) until plants reached the six-leaf growth stage, after which there was better control of liverseed grass. In 2004, better control of barnyardgrass was obtained at the two-leaf stage; however, there were no differences at the later growth stage. Similar results were obtained in the 2005 season.

6.3.1 Barnyardgrass response

The percentage of barnyardgrass plants surviving glyphosate application decreased as the dose of glyphosate increased. The response of barnyardgrass to increasing rates of glyphosate is shown in Figure 6.1.

In all years and growth stages, the effect of dose rate on the percentage of survivors was highly significant (Appendix 6.2). The impact of growth stage on survival under glyphosate application was also significant. The interaction between glyphosate dose rate and plant growth stage was significant in 2005. The highest rate of 1035 g ha^{-1} glyphosate provided over 95 percent control of barnyardgrass in all years and growth stages, and in 2004 and in 2005 control was 100 percent in all situations. At 690 g ha^{-1} glyphosate, over 95 percent control was obtained in all years and situations with the exception of the two-leaf application in 2003. At the two-leaf stage in 2005, the lowest rate of 86 g ha^{-1} glyphosate controlled almost 80 percent of barnyardgrass plants, while at the six-leaf stage less than 20 percent of plants were controlled by this rate.

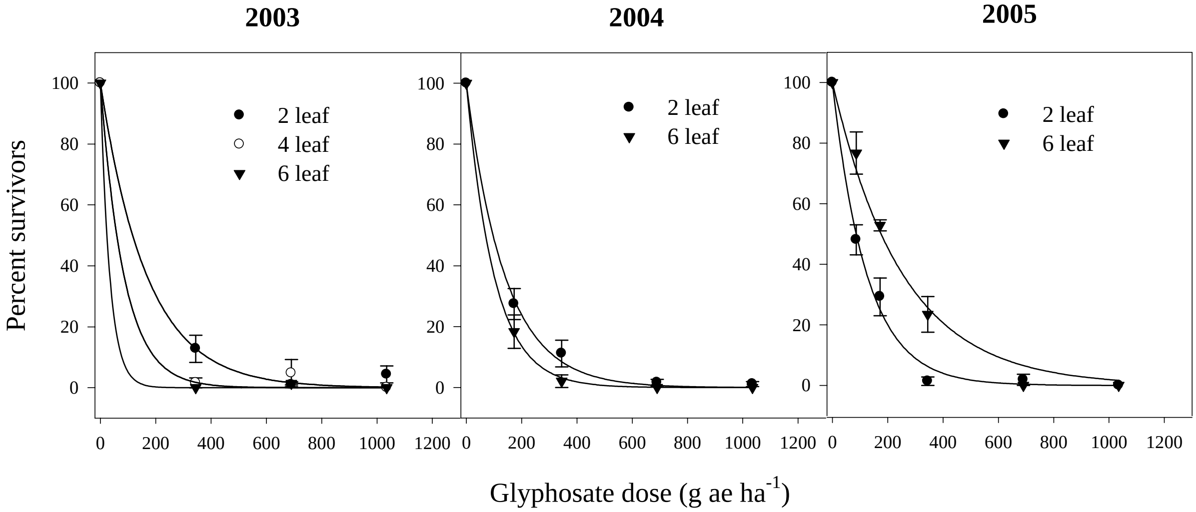
Figure 6.1 Dose mortality-response of barnyardgrass to increasing rates of glyphosate in 2003, 2004 and 2005. Regression parameters are listed in Appendix 6.3. Bars indicate standard error of the mean.



6.3.2 Liverseed grass response

As glyphosate dose increased, the percentage of liverseed grass survivors decreased for all years and growth stages. The response of liverseed grass to increasing rates of glyphosate is shown in Figure 6.2. In all years and growth stages, the effect of glyphosate dose on the percent control was significant, as was the impact of growth stage. The interaction between dose rate and growth stage was significant in both 2003 and 2005, but not in 2004. At 1035 g ha^{-1} glyphosate, over 95 percent control was provided in all years and growth stages. Full control of liverseed grass was achieved at 1035 g ha^{-1} glyphosate in 2003 at the 2, 4 and 6-leaf stages. In 2004, both 1035 and 690 g ha^{-1} glyphosate controlled liverseed grass at the six-leaf stage for. Total control was also achieved at 1035 g ha^{-1} glyphosate at both growth stages in 2005 and for the 690 g ha^{-1} rate at the two-leaf stage in 2005. In 2005, the lowest rate of 86 g ha^{-1} failed to control more than 25 present of the liverseed grass plants at the six-leaf growth stage.

Figure 6.2 Dose mortality-response of liverseed grass to increasing rates of glyphosate in 2003, 2004 and 2005. Regression parameters are listed in Appendix 6.3. Bars indicate standard error of the mean.



6.4 DISCUSSION

This experiment has shown that glyphosate is an effective herbicide for the control of both barnyardgrass and liverseed grass. The impact of growth stage on survival under herbicide application was highly significant for both species in 2005 and also significant in previous years, although this effect was not as pronounced. The reasons for this are unknown; however, they are most likely related to weather conditions. In 2005, there was frequent rainfall throughout the experiment, possibly enabling the older plants to recover better from the effects of the herbicide at the lower dose rates. However, once the dose rate applied reached 690 g ha^{-1} glyphosate (equivalent to 1 kg ha^{-1} of Roundup Ready® herbicide), full control was obtained at both growth stages for both barnyardgrass and liverseed grass. At a dose of 345 g ha^{-1} glyphosate applied in this experiment, the worst control of barnyard grass was 73 percent at the six-leaf stage in 2005 and for liverseed grass 77 percent at the six-leaf stage in 2005. At the two-leaf stage, greater than 87 percent control was achieved for both species in all years. These experiments have shown that good control of the two species can be achieved if glyphosate rates are kept above 690 g ha^{-1}.

The levels of control achieved by glyphosate in this experiment were similar to those reported by Koger *et al*. (2005), who obtained 99 and 93 percent control at the 2- to 3-leaf and 5- to 6-leaf respectively, by 840 g ha^{-1} glyphosate. Glyphosate control of barnyardgrass in experiments conducted by Lanie *et al*. (1993) was lower than that observed in this experiment and in experiments conducted by Koger *et al*. (2005). Lanie *et al*. (1993) achieved 71% control of barnyardgrass at glyphosate rates of 840 and 1120 g ha^{-1}, and 86% control at 1680 g ha^{-1} 14 days after treatment. The level of control decreased at 28 days after treatment (51, 54 and 67 percent, respectively). Reasons for these differences are most likely due to non-irrigated conditions and the variability in growth stage of barnyard grass plants treated by Lanie at al. (1993), which ranged from 5 to 38 cm. Plants that have reached these heights have mostly likely tillered and were therefore older than plants treated in this experiment.

Control of liverseed grass was more effective in this study than reported by Felton *et al*. (1990). Smaller plant size and irrigated conditions that prevented the liverseed grass plants from becoming moisture stressed probably contributed to the higher levels of control for liverseed grass in the current study.

6.4.1 Herbicide resistance implications

As previously stated, the survey of growers conducted in Chapter 3 revealed that the majority of growers apply higher rates of glyphosate to weeds present in cotton fields. This experiment showed that even at a rate of 1 kg ha^{-1} of Roundup Ready® herbicide (equating to 690 g ha^{-1} of glyphosate), mortality of barnyard grass and liverseed grass plants was never below 87 percent. These high rates of glyphosate impose a strong selection pressure on weeds causing high rates of mortality (Jasieniuk *et al*. 1996). Plants can only adapt if resistance genes are present in a population and there is a sufficiently large enough phenotypic effect that allows the survival of some individuals. Polygenic inheritance requires recombination among individuals for many generations to bring together enough favourable alleles to produce a highly resistant phenotype. Therefore, polygenic inheritance is more likely to occur when there is a weak selection pressure imposed that would occur with sublethal doses and a larger number of plants surviving each application (Jasieniuk et al. 1996). Therefore, it is most likely that resistance evolution will be the result of a major single gene, rather than the additive effect of smaller genetic effects (Jasienuik *et al*. 1996). As a result it will be assumed in the glyphosate resistance model in Chapter 7 that resistance is due to a major single gene.

CHAPTER 7

PREDICTING THE RATE OF GLYPOSATE RESISTANCE EVOLUTION IN GRASS WEEDS IN GLYPHOSATE-RESISANT COTTON

7.1 INTRODUCTION

Since the introduction of glyphosate-resistant cotton into Australia in 2000, this new management tool has become an important management option in cotton. However, it also has the potential to greatly alter management in favour of a heavy reliance on glyphosate. This may place the industry at risk of glyphosate resistance evolution in weed species should growers choose to use glyphosate in substitution of, rather than addition to, existing weed management practices (Roberts 1999). The survey conducted in Chapter 3 to investigate changes that had taken place in weed management until now showed that management had slightly changed in favour of glyphosate. Although the changes were only marginal, the temptation to use glyphosate-only management is still apparent. The looming introduction of enhanced glyphosate-resistant technology will increase the risks of glyphosate resistance evolution.

The complexity of biological processes that influence the evolution of herbicide resistance dictates a focus on the interaction between life history processes and population genetics. Computer models can provide a tool for evaluating management tactics and provide the opportunity to focus on the interaction between life history processes and population genetics (Maxwell *et al*. 1990). A number of models simulating the population dynamics and herbicide resistance of weed populations have been developed (Gressel and Segel 1978; Maxwell *et al*. 1990; Gardner *et al*. 1998, Cavan *et al*. 2000, Neve *et al*. 2003). As weed management in Australian cotton fields has potential to shift from a more integrated approach to one more heavily reliant on glyphosate, the development of glyphosate-resistant weeds is an emerging threat to the sustainability of the industry. The model presented here was used to explore the possible effects of these changing management practices in the cotton industry.

In previous chapters, the characteristics of barnyardgrass and liverseed grass have been studied in terms of their biological processes and responses to management practices in a glyphosate-resistant cotton cropping system. These two species will now be used to investigate the effect of different management practices routinely used in the Australian cotton industry on the evolution of herbicide resistance. Both weed species can germinate in high numbers, are currently easily controlled by glyphosate, produce large amounts of seed and are highly competitive with cotton (Maun and Barrett 1986; Keeley and Thullen 1991; Roberts 1990; Adkins et al. 1998; Norris et al. 2001). These biological characteristics make them worthy candidates for investigation of glyphosate resistance evolution.

7.2 MATERIALS AND METHODS

7.2.1 The Model

The model is based on the systems experiment in Chapter 4. It is broken up into five separate submodels, each reflective of the five treatments in the systems experiment. The treatments were as follows:-

1. Roundup Ready herbicide only (RR Only),
2. Roundup Ready herbicide plus a combination of conventional weed management practices (RR + IWM),
3. Roundup Ready herbicide plus a reduced residual herbicide program (RR + Res.),
4. Roundup Ready herbicide plus a grass herbicide (RR + Grass),
5. A combination of conventional weed management practices only (IWM Only).

A more detailed description of each treatment is given in Chapter 4 and Appendix 4.1.

The model replicated a simulated field size of 50 ha and assumed that the population within the field was finite. It also assumed that the populations were closed, i.e. there was no gene flow into the field from surrounding populations. As a result of assuming finite populations, extinction of resistance genes may occur when the frequency of resistance alleles is lower than the overall population density as described by Diggle *et al*. (2003). Extinction of alleles occurs at a point where the density of plants in the given field is less than one, and therefore unable to produce seed.

7.2.2 Initial frequency of glyphosate resistance alleles

The initial frequency of the resistance allele has an important impact in determining the timeframe over which resistance will evolve. Currently, the initial frequency of glyphosate resistance is not known for these two species. Such frequencies are very difficult to measure in unselected wild populations even for species that do have glyphosate resistance, indicating that mutations conferring resistance are extremely rare (Padgette *et al*. 1995; Jander *et al*. 2003; Neve *et al*. 2003). This model therefore applied frequencies similar to those simulated for glyphosate resistance in rigid ryegrass as in Neve *et al*. (2003) of 1×10^{-8} and 1×10^{-6}. It is also likely that prior use of glyphosate may have increased the frequency of glyphosate resistance alleles in populations prior to the introduction of glyphosate tolerant cotton. Therefore, the initial frequency of resistance may be variable between farms and fields depending on previous history.

7.2.3 Genetic aspects

For the purposes of the model, the genetic properties of the two model weed species were assumed to be such that resistance is nuclear, dominant and conferred by a single gene. This assumption was based on the fact that, in most cases studied, a single gene confers herbicide resistance (Darmency 1994). In addition, glyphosate resistance in both rigid ryegrass and Canadian fleabane (*Conyza canadensis* (L.) Cronq.) is inherited as an incompletely dominant nuclear-encoded trait conferred by a single gene (Lorraine-Colwill *et al*. 2001; Zelaya *et al*. 2004). As a result of the assumption that resistance is nuclear, dominant and conferred by a single gene, the probability of survival for all homozygous resistant alleles $P(S_{RG})$ is set at 100% in the model. Based on previous experiments on F_1 crosses of susceptible and resistant rigid ryegrass plants (Tardiff *et al*. 1996; Lorraine-Colwill *et al*. 2001) the probability of survival for heterozygous alleles $P(S_{RrG})$ is also assumed to be high under normal glyphosate use rates.

The initial frequencies of the homozygous resistant (RR), heterozygous (Rr) and homozygous susceptible (rr) genotypes are initially assumed to be in Hardy-Weinberg equilibrium, and frequencies are subsequently determined based on the levels of self-fertilization and out-crossing.

Barnyardgrass plants are highly autogamous, and their mating system involves a high degree of self-fertilization, with a small amount of out-crossing by wind (Maun and Barrett 1986). Mating characteristics of liverseed grass are not known; however, close inspection of flowers (Plates 7.1 and 7.2), which are exposed from the floret, suggests a slightly higher level of out-crossing than barnyard grass, although some self-fertilization appears to occur as bagged spikes still produce seed. Mating is assumed to be random between genotypes for the proportion of plants that are out-crossing.

Plate 7.1 Microscope view of barnyardgrass florets illustrating the degree to which stamen and stigma are exposed.

Plate 7.2 Microscope view of liverseed grass florets illustrating the degree to which stamen and stigma are exposed.

7.2.4 Germination characteristics

Germination probabilities were calculated from data collected from the systems experiment in Chapter 4. For each species, germination probabilities were separated into two main groups. These were treatments that did not receive soil-applied residual herbicides and those that did. Germination of barnyardgrass tended to be scattered throughout the season, but was concentrated into five main cohorts. The majority of liverseed grass germinated early in the season in two main cohorts, with a very small number of later germinations occurring through to mid-season. The probabilities for the germination of each cohort were described using the following function in Microsoft Excel. This function returns a random number between the highest and lowest numbers specified. Parameters for germination probabilities are listed in Tables 7.1 and 7.2.

$$P(G_{CN}) = \mathrm{RANDBETWEEN}(P(G_{CNlow}),P(G_{CNhigh}));\ \text{where} \tag{7.1}$$

$P(G_{CN})$ = probability of germination for cohort N.

$P(G_{CNhigh})$ = highest probability of germination for cohort N obtained from the field data.

$P(G_{CNlow})$ = lowest probability of germination for cohort N obtained from the field data.

Table 7.1. Barnyard grass germination probabilities applied to individual cohorts within the model (based on systems experiment data in Chapter 4).

	Residuals		**No Residuals**	
Cohort (N)	**$P(G_{CNhigh})$**	**$P(G_{CNlow})$**	**$P(G_{CNhigh})$**	**$P(G_{CNlow})$**
1	0.054	0	0.33	0.003
2	0.080	0	0.26	0
3	0.013	0	0.26	0
4	0.054	0	0.11	0
5	0.00053	0	0.02	0

Table 7.2. Liverseed grass germination probabilities applied to individual cohorts within the model (based on systems experiment data in Chapter 4).

	Residuals		**No Residuals**	
Cohort (N)	**$P(G_{CNhigh})$**	**$P(G_{CNlow})$**	**$P(G_{CNhigh})$**	**$P(G_{CNlow})$**
1	0.01	0	0.68	0.003
2	0.01	0	0.11	0
3	0.009	0	0.16	0

7.2.5 Weed control impacts

The effect of residual herbicides is taken into account within the probability of weeds germinating. For the RR + Res. treatment, a residual application only occurred as a lay-by application mid-season. Therefore, the germination probabilities for the first cohort are taken from the "No Residuals" parameters and then from the "Residuals" parameter thereafter.

Probabilities of survival for glyphosate susceptible alleles $P(S_{rGN})$ were estimated from a exponential decay function describing dose-mortality response experiments conducted in the field (Chapter 6).

$$P(S_{rGN}) = Surv_{MAX}*EXP^{(-0.0053*GlyDoseN)}\text{; where} \qquad (7.2)$$

SurvMAX = Maximum survival of weeds sprayed.
GlyDoseN = Dose of glyphosate applied at time N.

Roundup Ready® technology allows a maximum of two x 1.5 kg over-the-top applications that must occur before the four-leaf stage of cotton, with a following 1.5 kg application by a directed/shielded sprayer before canopy closure (Monsanto 2000). It is also common practice to use glyphosate as a knockdown herbicide concurrently with residual herbicide applications before the cotton is planted for both Roundup Ready® or conventional cotton production systems. These practices are reflected in the model, with an initial knockdown application of glyphosate occurring in all treatments.

Other weed control methods used are assumed to have equal effect on both resistant and susceptible alleles. In the RR + grass and IWM only treatments, the probability of survival of the two species to applications of haloxyfop is represented by $P(S_H)$. This parameter was measured from observations in the systems experiment (Chapter 4). It is assumed that no haloxyfop resistance evolved in the model. When the lay-by herbicide, in this case Prometryn $P(S_P)$, is applied with a wetting agent, it was also assumed to have the same effect on the probability of weed survival for all genotypes. For the IWM treatments (RR + IWM and IWM only), inter-row cultivation or tillage is included. The probability of survival from tillage $P(S_T)$ is directly related to the percentage area covered by the tillage equipment. In the systems, experiment the implement used covered 70% of the area between the cotton rows.

7.2.6 Seed production

The amount of seed produced by surviving liverseed and barnyard plants was determined by competition studies between the weed species and cotton in (Chapter 5). The cotton density is kept constant in the model at 12 plants per meter of row, with the following hyperbolic equation being adapted from Norris *et al*. (2001).

$$\mathrm{Seed}_A = (\mathrm{Seed}_{MAX} * \mathrm{Dens}_A)/(\Delta \mathrm{Seed}_A + \mathrm{Dens}_A);\ \text{where} \qquad (7.3)$$

Seed_A = Seed produced per meter for each respective allele.
Seed_{MAX} = Maximum seed production.
Dens_A = density per meter of each respective allele
$\Delta\mathrm{Seed}_A$ = Change in seed production.

The competition experiment and the systems experiment mentioned above and Keeley and Thullen (1991) all indicated established cotton was very competitive against grass weeds, especially after canopy closure. Thus it was assumed the final weed cohort germinating was unable to produce seed.

The biological fitness of resistant allele for the two model species is not known. These species currently do not have resistance to glyphosate and the fitness penalties of biotypes resistant to other herbicides have not yet been fully elucidated. It is reasonable to assume that there would be some fitness cost to resistance alleles in the absence of glyphosate, based on early studies of triazine resistance (Jasieniuk *et al*. 1996). Many-triazine resistant alleles were found to have impaired photosynthesis resulting in a fitness cost of between 42% and 70% biomass reduction when compared to susceptible alleles (Jordan 1999). To gain a complete understanding of fitness effects, they should be measured at all stages of the life cycle (Diggle and Neve 2001; Vila-aiub *et al*. 2005). However, due to these factors not being known, a fitness penalty Fp_R for resistant and Fp_{Rr} for heterozygous individuals has been applied as a percentage reduction to the seed producing capabilities of both genotypes (Appendix 7.1). The effect of changing fitness penalties on resistance evolution was also examined.

7.2.7 Seedbank characteristics

The initial seed bank, Sbank_i, is split into a separate seed bank for each genotype Sbank_A. Seed produced in year n of each genotype (Seed_A) less the total germinations from all cohorts were added to the seed bank for the start of that year. Factors such as seed removal by insects, and survival in the soil were incorporated by an exponential decay function that, in the absence of any data, assumed that approximately half the amount of viable seed in the soil from the previous year remains for the next year.

$$\mathrm{Sbank}_{An+1} = (\mathrm{Seed}_A + \mathrm{Sbank}_{An} - \sum \mathrm{Germ}_{ACN}) * \mathrm{EXP}^{(-0.6931*\mathrm{Yrn})};\text{ where} \qquad (7.4)$$

Sbank_{An+1} = starting seedbank with respect to each genotype for the next year.
Sbank_{An} = seedbank for each genotype at start of current year.
Germ_{ACN} = Germinations for each cohort of each genotype.
Yr_n = number of years that the seed lot each year has remained in the seedbank.

7.3 RESULTS

8.3.1 Weed management influences on glyphosate resistance

Resistance evolution in relation to the five weed management treatments was simulated over 30 years for two initial resistance frequencies of 1×10^{-8} and 1×10^{-6} for both species (Figures 7.1 and 7.2). Default values for parameters are listed in Appendix 7.1. At the lower resistance frequency, resistance evolved (determined as when the frequency of the resistant allele reached 0.5) in the RR Only treatment after 12 years for barnyard grass and 10 years for liverseed grass with the frequency of resistant alleles reaching 0.8 at 17 years for both species. However, for the higher initial frequency of resistance at 1 x 10^{-6}, the timeframe of resistance for the RR Only treatment was reduced to approximately 8 years for both species.

Figure 7.1. Cumulative probability distributions for predicted rates of glyphosate resistance evolution for barnyard grass and liverseed grass under the 5 weed management regimes investigated. Initial frequency of resistant alleles set at 1x10^{-8}. Note that all curves are represented; however, resistance evolved only in the RR Only treatment.

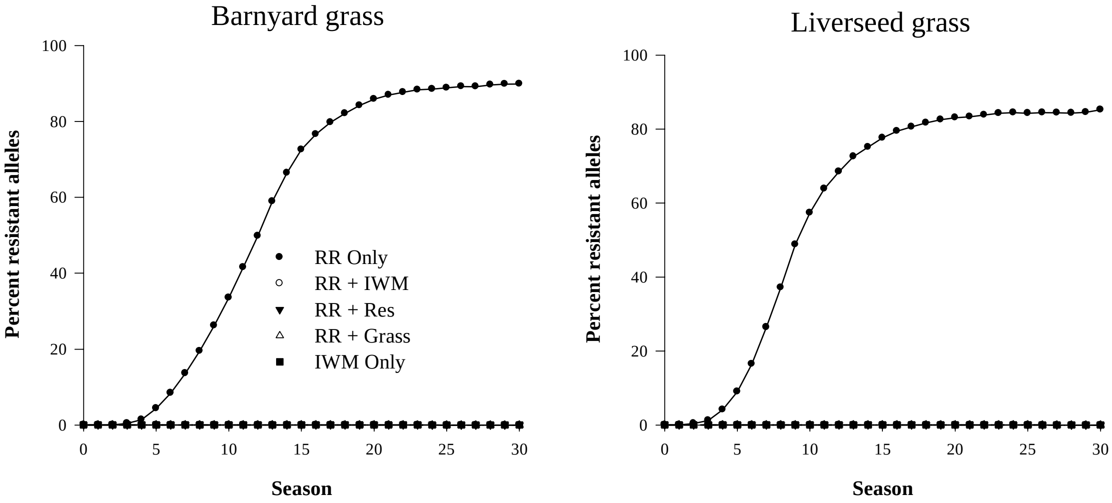
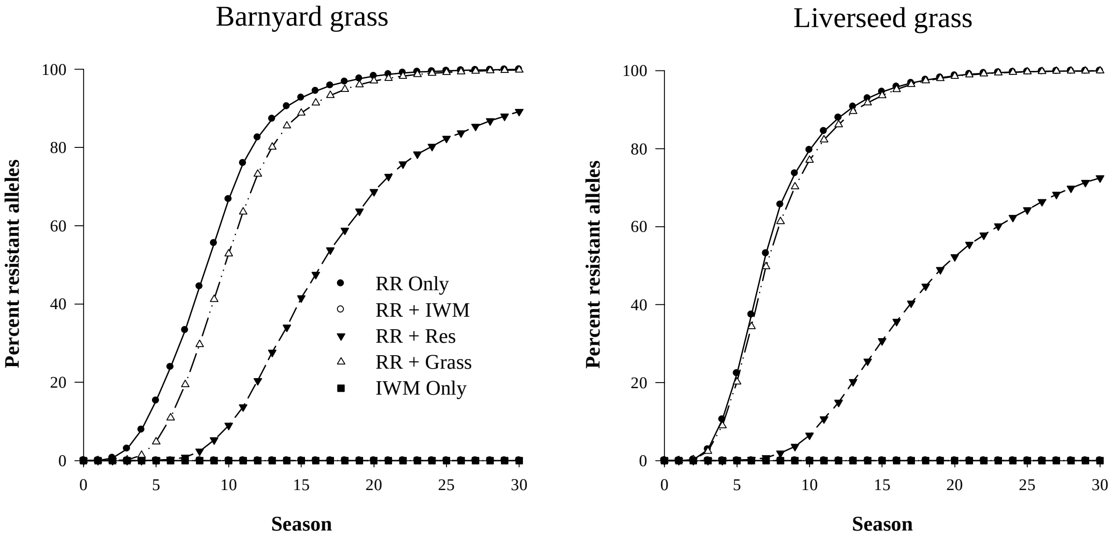


Figure 7.2. Cumulative probability distributions for predicted rates of glyphosate resistance evolution for barnyard grass and liverseed grass under the 5 weed management regimes. Initial frequency of resistant alleles set at 1x10^{-6}.



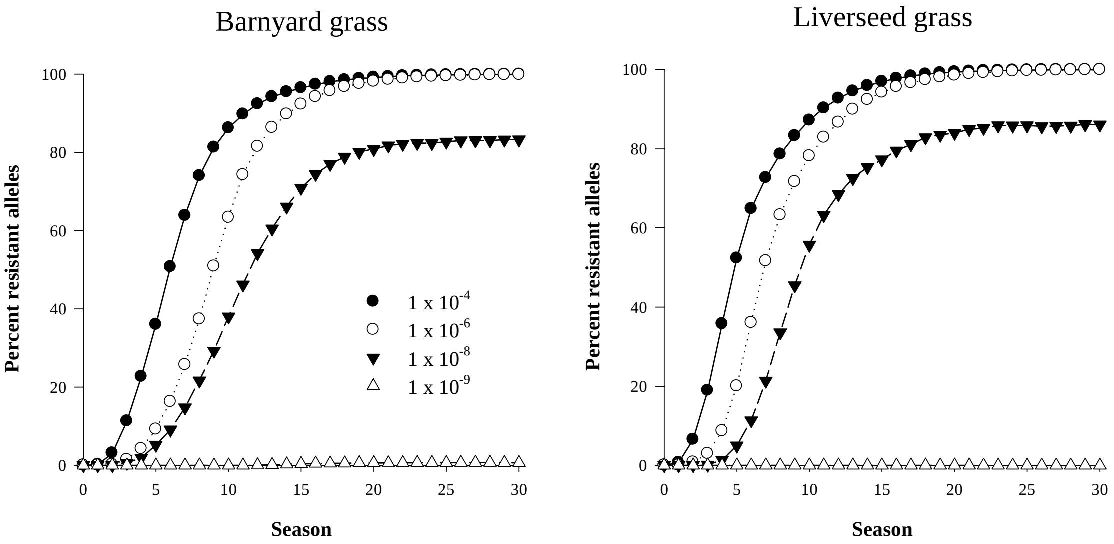
In these simulations, resistance failed to evolve in all other treatments when the initial frequency of resistance was set at 1 x 10^{-8}. However, when the initial frequency of resistance was set at 1 x 10^{-6}, resistance evolved in the RR + Res. and RR + Grass treatments as well as the RR Only treatment. Using a grass herbicide in addition to glyphosate only delayed the onset of resistance by 1 year for barnyardgrass, and had no effect on resistance evolution in liverseed grass when the initial frequency of resistance was set at 1 x 10^{-6}. The grass herbicide was only applied once each season and there was still a heavy reliance on glyphosate in this treatment. When the initial frequency of resistance was lower (10^{-8}), this treatment was effective at delaying glyphosate resistance; however, the model did not take into account the possibility of resistance evolving to haloxyfop-methyl.

The reduced residual treatment (RR + Res.) was also effective at delaying resistance, especially when the initial frequency of resistance was lower. With an initial frequency of resistance alleles set at 1 x 10^{-6}, resistance was delayed by 7 years for barnyardgrass and over 10 years for liverseed grass compared to the RR Only treatment.

7.3.2 Initial frequency of glyphosate resistance alleles

A sensitivity test was conducted for different values of the initial frequency of resistance alleles. This showed that the initial frequency of resistance alleles strongly influenced the timeframe of resistance evolution. The results of a series of simulations are shown in Figure 7.3. A 100-fold decrease in the initial frequency of resistance alleles from 1 x 10^{-4} to 1 x 10^{-6} delayed resistance evolution by three generations, as did a change in the initial frequency of resistance alleles from 1 x 10^{-6} to 1 x 10^{-8}. The simulation indicated that at initial frequencies of less than 1 x 10^{-9} resistance would not evolve. This result was a function of field size and initial seedbank numbers. With a smaller area and lower seedbank density, there was less chance of finding a resistant individual in the population.

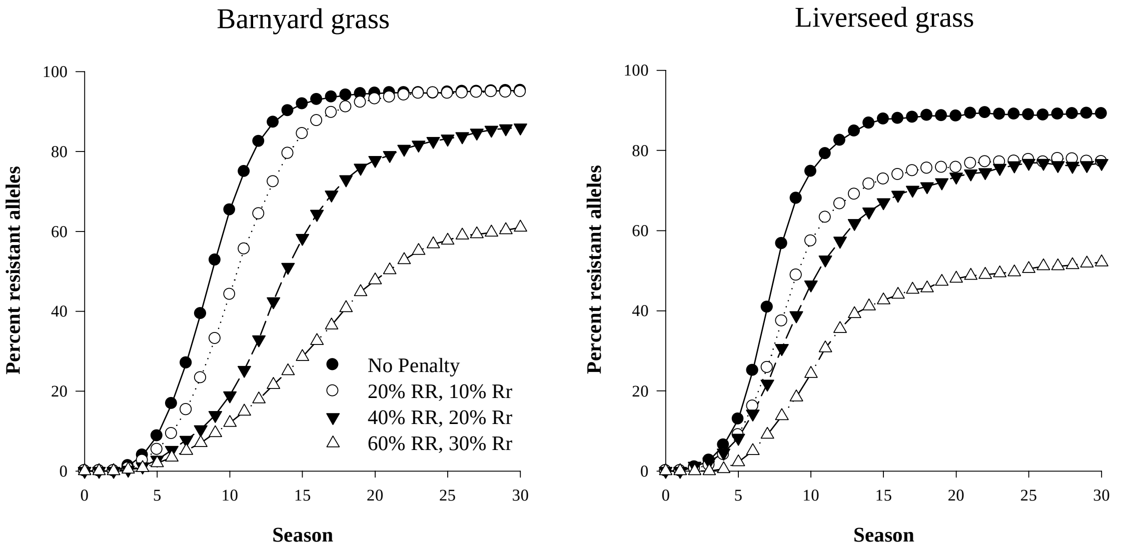
Figure 7.3. Cumulative probability distributions for predicted rates of glyphosate resistance evolution for barnyard grass and liverseed grass for the RR Only treatment for a range of initial resistance frequencies.



7.3.3 Fitness penalties and resistance evolution

A fitness penalty associated with a resistance allele is likely to reduce the frequency of resistance in populations when the herbicide in not used (Jasienuik *et al*. 1996; Jordan 1999). The influence of a fitness cost on the predicted evolution of glyphosate resistance in the two species was examined in a sensitivity test. This showed that fitness of the resistance allele could have a major effect on resistance evolution. When there was no fitness cost, resistance occurred in 9 years for barnyardgrass and in 8 years for liverseed grass (Figure 7.4). With a fitness cost of 20 percent for resistant individuals and 10 percent for heterozygous individuals, resistance evolution was delayed by 2 years for barnyardgrass and by 1 year for liverseed grass. Higher fitness costs had greater impact on the evolution of resistance. For a fitness cost of 60 percent for homozygous resistant individuals and 30 percent for heterozygous individuals, resistance was delayed until 21 years for barnyardgrass and 25 years for liverseed grass. Therefore, major fitness penalties on resistance alleles can significantly delay the evolution of resistance.

Figure 7.4. Cumulative probability distributions for predicted rates of glyphosate resistance evolution for barnyard grass and liverseed grass for the RR Only treatment for a range of fitness penalties. Initial frequency of resistant alleles is $1x10^{-8}$.



With respect to rigid ryegrass, Neve *et al*. (2003) suggested that the most likely fitness penalty was around 25 percent for homozygous resistant and 10 percent for heterozygous phenotypes because it closely matched the timeframe for actual resistance evolution in that species. With fitness penalties of this order, resistance is likely to evolve in both barnyardgrass and liverseed grass should glyphosate be the only weed control used in cotton. While fitness penalties may delay the evolution of resistance in the RR strategy, they are probably even more beneficial where additional weed control options are chosen.

7.4 DISCUSSION

Glyphosate use in glyphosate tolerant cotton has become an important tool in weed management in cotton crops in Australia. It allows conservation tillage practices, reduces reliance on residual herbicides and gives growers the option of a broad-spectrum, post-emergent herbicide in crop (Jones and Snipes 1999; Faircloth *et al*. 2001). However, this modelling exercise has shown the importance of not abandoning conventional weed control methods in glyphosate tolerant cotton in order to use glyphosate alone. The model predicted glyphosate resistance evolution after 12 years for barnyard grass and 17 years for liverseed grass when glyphosate is used alone. Neve *et al*. (2003) reported that in Australia, in some cases, resistance evolved in *Lolium rigidum* following 15 years of applications of glyphosate. However, in many agricultural and horticultural situations worldwide, glyphosate had already been applied over 15 times to weed populations without the evolution of resistance. Computer simulations give scientists the ability to examine possible reasons for these contradictory findings by generating a range of scenarios that are difficult to test by experimentation.

The simulations conducted highlight the importance of an IWM approach to weed management for resistance prevention. The RR + IWM and IWM only were the only treatments where resistance did not evolve. These treatments reduced the selection pressure for resistance alleles and randomly caused allele extinction. The timeframes of resistance evolution simulated here could be considered conservative due to the high number of applications of glyphosate that occur in a Roundup Ready® cotton system, compared to southern Australian cropping systems.

Neve *et al*. (2003) suggested that 100 percent of populations would evolve resistance after 10 years of selection when the initial resistance frequency was 1 x 10^{-6} and after 25 years for an initial frequency of 1 x 10^{-8}. Selection pressure plays an important role in the evolution of resistance when one or few herbicides are used continually over a period of time (Preston and Reiger 2000; Maxwell and Mortimer 1994). In practice, rigid ryegrass evolved glyphosate resistance after 2 to 3 applications of glyphosate per year over 15 years (Powles *et al*. 1998), and *Eleusine indica* after 6 to 8 applications of glyphosate per year over 4 years (Lee and Ngim 2000). In the latter case, there are likely to have been more than one generation of weeds per year. In glyphosate tolerant cotton, glyphosate is applied up to four times within the cotton growing season. Hence, there is reason to assume that glyphosate resistance could evolve earlier if glyphosate is the only management technique used.

The initial frequency of resistance alleles had a profound effect on the timeframe for resistance evolution. Decreasing the initial frequency of resistance alleles delayed resistance evolution. This result has impacts at a field scale where the number of resistant plants present is a function of the initial frequency of resistant alleles and the density of weed populations. For rare alleles, there is less chance of finding a resistant individual in the population when there is a smaller seed bank density. This emphasises the importance of keeping weed numbers lower to minimise chance events (Christoffers 1999; Diggle *et al*. 2003). However, due to the random stochastic nature of plant populations, resistance alleles may still be present even at low weed densities. Therefore, management practices that employ a range of weed management tactics are crucial in ensuring that glyphosate resistance alleles can be driven to extinction, rather than being selected by continuous glyphosate applications.

Apart from management practices, resistance evolution can be significantly enhanced or delayed, depending on a range of biological factors. In this chapter, sensitivity testing showed that initial resistance gene frequencies and fitness penalties had a major effect on the time frame of resistance evolution. The sensitivity testing reported here indicated that the initial resistance frequencies are important to consider because, when combined with other factors, the number of generations for resistance to evolve in a weed population is significantly reduced (Jasieniuk *et al*. 1996).

This model has given an approximate timeframe of resistance evolution on the basis of measured population dynamic characteristics, with unknown values for initial resistance frequencies and fitness penalties being estimated by extrapolation (Christoffers 1999). Despite this, it is accepted that reliance on glyphosate (or any herbicide) increases the danger of resistance evolution, almost regardless of initial resistant gene frequencies and fitness penalties. The simulations in this model showed that including IWM practices into weed control is the best way to prevent such resistance evolution. Weed control strategies must complement each other so that plants surviving a herbicide treatment are controlled by another treatment, be it mechanical or via a different herbicide mode of action (Christoffers 1999). Therefore, strategies to prolong the use of glyphosate and the advantages of glyphosate-resistant cotton must incorporate a range of weed management options.

CHAPTER 8

GENERAL DISCUSSION

8.1 INTRODUCTION

The introduction of glyphosate-resistant cotton, into Australia brings a number of advantages in providing a broad-spectrum, post-emergent herbicide that can be applied to weeds without damaging the crop. However, the threat of glyphosate resistance evolving in weeds is increased in glyphosate-resistant cotton, as multiple glyphosate applications in crop each season are now possible. Weed control practices need to be reconsidered over the long term so that the possibilities of resistance evolution and weed shifts can be determined. The evolution of glyphosate resistance in rigid ryegrass (*Lolium rigidum*) in southern Australia and in other species around the world illustrates the risks involved.

This thesis attempts to define the risks of the evolution of glyphosate resistance from the introduction of glyphosate-resistant cotton. In order to do this, the effect of the introduction of glyphosate tolerance on weed management practices in the cotton industry was examined via a grower survey comparing weed management in glyphosate-resistant and conventional cotton fields. The population dynamics of two important grass weeds, *Echinochloa crus-galli* (barnyardgrass) and *Urochloa panicoides* (liverseed grass) in a glyphosate-resistant cotton system was also studied. Data from these experiments formed the basis for a glyphosate resistance model to determine the effect of management practices on the potential for resistance evolution in these two species in the long term. This information will provide a framework for resistance prevention for a range of weeds in a glyphosate-resistant cotton system.

8.2 CURRENT PRACTICES AND HERBICIDE USE IN GLYPHOSATE-RESISTANT AND CONVENTIONAL COTTON FIELDS

In Chapter 3, a survey of growers was conducted to determine how weed management practices were changing with the introduction of glyphosate-resistant cotton. This survey was able to make comparisons between glyphosate-resistant and conventional cotton fields with regard to weed species and prevalence, weed management practices and herbicide use in order to determine the impact glyphosate-resistance has had on cotton growing. At the time of the survey, glyphosate tolerance had been in use in cotton systems for three years. Glyphosate resistance had received a rapid adoption, with 40 percent of growers in the industry growing glyphosate-resistant cotton (S. Ainsworth, Monsanto, pers. comm.). Since then the adoption of glyphosate-resistance has increased to over 70 percent of the total cotton area planted (G. Constable, CSIRO, pers. comm.)

While growers reported no changes in weed species in glyphosate-resistant compared to conventional fields, slight changes to management practices had taken place. With regard to weed species, there were a number of growers who were targeting problem weeds, such as *Cyperus rotundus* and *Hibiscus trionum,* with glyphosate applied over-the-top of cotton. This created an additional option for management of these weed species. In the majority of cases, growers felt they obtained better overall control of weeds in glyphosate tolerant cotton fields. However, *Cyperus rotundus* and other weeds, such as *Ipomea lonchophylla*, *Polymeria pusilla*, *Physalis minima*, and *Rhyncosia minima,* still proved to be a problem for some growers. Nearly all growers reported that the window for over-the-top applications was too narrow, because the four-true leaf stage of the cotton crop was reached within a few weeks of crop emergence.

The greatest change in weed management and herbicide use was the increase in glyphosate applications. Although there were only two additional applications of glyphosate per season, the total amount of glyphosate applied increased dramatically in glyphosate-resistant cotton fields. These two applications were over-the-top applications, an advantage of using glyphosate-resistant cotton.

There was also a decrease in the amount of residual herbicides used by growers. However, the majority of growers were still using residual herbicides at some time during the season. The changes observed in this survey were similar to those observed in a survey of glyphosate-resistant cotton growers in Australia conducted by Doyle *et al*. (2003) and surveys of farmers in other glyphosate-resistant crops in the United States and Canada (Lin *et al*. 2001; Serecon and Koch Paul 2001). An increase in glyphosate use was also observed in glyphosate tolerant soybeans in the United States (Lin *et al*. 2001). In irrigated fields, such as those included in the survey reported in this study, inter-row cultivation was still seen as an important practice, although this was mainly for bed formation and water flow rather than weed control. Although weeds in the plant line aren’t controlled by inter-row cultivation, it will be an important feature in glyphosate-resistant cotton systems enabling another form of weed control between the rows.

Substitution of glyphosate for other herbicides and weed management options leading to the evolution of glyphosate resistant weeds is the main concern in glyphosate-resistant cotton (Roberts 1999). While such a substitiution was not evident at this stage with current glyphosate tolerant technology, the introduction of enhanced glyphosate resistance with Roundup Ready Flex® cotton creates an increased potential for substitution of glyphosate to occur. The growers surveyed envisaged a reduction of 30% in the number of IWM practices that would be practiced in Roundup Ready Flex ® cotton compared to the current Roundup Ready ® cotton. While this may increase the selection pressure for the evolution of glyphosate resistance in the future, the current Roundup Ready® crop management plan stipulates that the technology must be managed carefully, with any escapes from glyphosate applications controlled by some other means and recommendations for a range of weed management practices in high weed pressure situations (Monsanto 2000). Future weed management plans for Roundup Ready Flex ® cotton will also need to include these features to ensure the effectiveness of glyphosate and prevent resistance evolution.

8.3 RESPONSE OF BARNYARDGRASS AND LIVERSEED GRASS TO A RANGE OF MANAGEMENT PRACTICES IN A GLYPHOSATE-RESISTANT COTTON SYSTEM

Chapter 4 examined weed management practices in a glyphosate-resistant cotton system in more detail. The treatments applied were designed to reflect practices emerging from the survey and currently in use in the cotton industry. The characteristics of barnyardgrass and liverseed grass were selected for study due to their germinating in high numbers, high fecundity (Keeley and Thullen 1989; Norris 1991; Veenendaal *et al*. 1996) and relative ease of control by glyphosate (Lanie *et al*. 1993; Norris *et al*. 1996). The effects of management practices on the population dynamics of these weed species were examined.

The treatments were graduated to reflect systems using glyphosate only through to a fully integrated system. All treatments used proved effective at reducing the number of weed seeds in the soil seed bank. As a result, there was a substantial reduction in number of barnyardgrass and liverseed grass plants germinating throughout the 3 years of the experiment. The benefits of using a soil-applied residual herbicide were apparent for those treatments that used them. These treatments (RR + IWM and IWM Only) had lower emergence for barnyardgrass and liverseed grass, resulting in greater reductions in the soil seed bank.

8.4 GROWTH AND SEED PRODUCTION OF BARNYARDGRASS AND LIVERSEED GRASS IN COMPETITION WITH COTTON

In order to construct a model to simulate the population dynamics and the potential of weed populations to evolve resistance, information on a number of factors is required. One important factor is the seed-producing capabilities of that species over a range of weed densities. The above ground biomass and seed production of barnyardgrass and liverseed grass were examined in Chapter 5. These experiments produced valuable information on the characteristics of these two species under irrigated cotton systems in Australian conditions and provided important data for use in simulating the long-term outcome of applying different management practices.

Both weed species proved to be very competitive against cotton when emergence occurred with cotton. Barnyardgrass produced large numbers of seed, up to 26,500 seeds plant^{-1} at the lower densities and 40 000 seeds m^{-1} of cotton row at high densities. Liverseed grass also had high seed production with 11,000 seeds plant^{-1} at a density of 0.5 plants m^{-1}, and almost 60,000 seeds m^{-1} of cotton row at higher densities. These experiments also demonstrated that the concept of using single-year economic thresholds as a decision tool with these weed species needs to be questioned, particularly if single plants allowed to go to seed produce a significant seed rain (Norris 2001). This also has implications for the evolution of resistance if seed rain is not prevented. If plants that have been exposed to glyphosate are allowed to produce seed and replenish the seed bank, this may increase the probability of resistance evolution.

These experiments also highlighted the importance of early season weed control in cotton. Planting date had a major effect on the ability of both weed species to produce seed. Keeley and Thullen (1991) found that if cotton could be kept weed-free for a period of 9 weeks it became competitive with barnyardgrass and dramatically reduced the ability of barnyardgrass to grow and produce seed. Bosnic and Swanton (1997) also observed established corn crops were highly competitive against barnyardgrass seed production. The maximum seed production of barnyardgrass was reduced to 12,000 seeds m^{-1} of row, and liverseed grass seed production declined to 2,500 seeds m^{-1} of row for plants that emerged 6 weeks after cotton. Weed seeds that were planted 12 weeks after the cotton was planted failed to emerge in this experiment. Therefore, management of these two weed species needs to provide effective control of weeds right up to canopy closure of the cotton crop to prevent a large amount of seed production. In addition to this, particularly with barnyardgrass that has a scattered germination pattern throughout the season (Keeley and Thullen 1989), good crop agronomy that provides a competitive crop can aid in weed control to prevent later-germinating cohorts producing seed.

8.5 THE EFFECT OF GLYPHOSATE DOSE ON SURVIVORSHIP OF BARNYARDGRASS AND LIVERSEED GRASS

The number of survivors in a weed population following herbicide applications is an important factor in population dynamics and evolution of resistance for a weed species in a cropping system. The effect of glyphosate application on barnyardgrass and liverseed grass survivorship as part of a glyphosate-resistant cotton cropping system was examined in Chapter 6. This experiment provided dose-mortality curves that formed an integral part of the glyphosate resistance model developed in Chapter 7.

Glyphosate was an effective herbicide for the control of both barnyardgrass and liverseed grass in this experiment. The impact of dose rate on percentage survivors was significant for both species and growth stages. Once the rate of glyphosate applied was above 690 g ae ha^{-1}, mortality of both species was never below 87 percent. This experiment showed similar control of barnyardgrass as that reported by Koger *et al*. (2005). The benefits of timing applications to control smaller plants were also highlighted as higher mortalities were achieved in the current study than those achieved by Lanie *et al*. (1993) with larger barnyardgrass plants. Higher levels of control of liverseed grass plants in the current study compared with that of Felton *et al*. (1990) was probably the result of smaller plant size and the lack of water stress.

8.7 MODELLING THE EVOLUTION OF GLYPHOSATE RESISTANCE IN TWO GRASS WEEDS

The main output of this thesis is a model to predict the likelihood of glyphosate resistance evolution in weeds from the use of glyphosate in glyphosate-resistant cotton crops and to determine the effect of additional weed management practices on resistance potential. Chapters 4 to 6 gathered information on the population dynamics of barnyardgrass and liverseed grass with respect to the effect of management practices on germination and survival, seed production and response to glyphosate application. Chapter 7 presents the model that combines all these factors together to gain an understanding of factors that are important in delaying or preventing glyphosate resistance evolution.

The model was based on the systems experiment used in Chapter 4. However, as the two species studied have not yet evolved glyphosate resistance, estimates of characteristics such as initial resistance gene frequencies, inheritance and fitness penalties were derived from species such as rigid ryegrass that have evolved resistance to glyphosate. Rigid ryegrass is frequently present in high densities and has a high degree of genetic variability. Its ease of control by glyphosate has lead to intense selection pressure (Neve et al. 2003). These characteristics are similar to the species use in this model, thus making rigid ryegrass an ideal species to take resistance characteristics from.

The effects of a number of parameters were examined in a sensitivity analysis. When the RR Only treatment was modelled, resistance evolved after 12 years for barnyardgrass and 10 years for liverseed grass when the initial frequency of resistance alleles was 1×10^{-8}. If the initial resistant allele frequency in the population was increased, the timeframe for resistance evolution decreased. The relative fitness of resistant plants was also an important factor in determining the rate of glyphosate resistance evolution. Each increase in fitness penalty of 20 percent delayed the onset of resistance by an average of 4 years for the RR Only treatment.

The greatest influence on glyphosate resistance evolution was management. An irrigated cotton system has a relatively wide range of weed control options available for use. When a fully integrated weed management program was employed using residual herbicides, glyphosate and other post-emergent herbicides for weed control, glyphosate resistance did not evolve in the simulations, regardless of initial resistant allele frequency. As IWM practices were gradually reduced, the potential for resistance evolution increased. The influence of residual herbicides on the rate of resistance evolution was observed in the RR + Res. treatment. This strategy proved to be effective in delaying the onset of resistance. However, it was still unable to prevent resistance evolution over the period of the simulation at the higher initial resistance allele frequency of 1 x 10^{-6}. Although the residual herbicides were effective at reducing the germination percentage of the weeds, weeds that did germinate were afterwards only exposed to glyphosate as a post-emergent weed control. This led to resistance evolving, albeit at a slower rate than for the RR Only treatment.

The addition of a grass herbicide to the glyphosate applications in the RR + Grass treatment had a slight effect in delaying resistance evolution. There was little impact because the weed population still received four applications of glyphosate per year in this treatment. When the number of glyphosate applications per season was reduced, the timeframe for resistance evolution in the simulation increased significantly (data not shown). It is worthwhile noting that this simulation model model does not take into account the possibility of resistance evolving to the other herbicides used such as haloxyfop-methyl. Resistance to grass or residual herbicides might accelerate the evolution of resistance to glyphosate, as where other herbicides are not used, or do not work, more pressure is placed on glyphosate for weed control. The simulations emphasise the need for a range of post-emergent weed control options in addition to the pre-emergent herbicides for effective resistance prevention.

8.8 CONCLUSIONS

The principles of Integrated Weed Management have been shown in this thesis to be valuable for the prevention of herbicide resistance. The greatest influence on the possibility and timeframe for glyphosate resistance evolution in a glyphosate-resistant cotton system is the weed management strategy chosen. Other factors, such as the initial resistant allele frequency, inheritance characteristics and fitness penalties, do have an effect on resistance evolution. However, as has been shown in Chapter 7, the degree to which IWM is adopted in a glyphosate-resistant system has a much greater effect. If IWM practices are reduced, the timeframe and likelihood for resistance evolution increases.

Glyphosate resistance is now an important tool for weed management in cotton and has been widely received by the cotton industry, as shown in Chapter 3. Glyphosate-resistant cotton enables the use of a broad-spectrum, post-emergent herbicide in the cotton crop (Jones and Snipes 1999, Faircloth *et al*. 2001). As Chapter 6 showed, glyphosate is a very effective herbicide on barnyardgrass and liverseed grass, providing high levels of control. The concerns raised by Roberts (1998*a*) regarding the detrimental effects of taking a glyphosate only approach to weed control in substitution of, rather than addition to, existing IWM practices in cotton (Charles *et al*. 1995) have certainly been supported by this thesis. Chapter 3 demonstrates there is little risk of this in Roundup Ready cotton. However, when enhanced glyphosate-resistant technology arrives in the form of Roundup Ready Flex®, IWM practices could further decrease and glyphosate resistance become a greater risk.

Soil-applied residual herbicides have been shown to be an important weed management tool in reducing the numbers of weeds germinating, especially in high weed pressure situations. The use of such herbicides reduces the number of weeds that are exposed to glyphosate during the season, reducing the chances of a resistance evolving by potentially killing any individuals that may carry a resistance allele (Christoffers 1999; Diggle *et al*. 2003). The systems experiment in Chapter 4 showed that a reduced residual program with glyphosate is an effective and economic method for weed control. When the long term impacts of this approach were examined in the resistance model, it was clear that the RR + res. treatment is only effective in delaying resistance evolution. However, it requires other post-emergent weed management options in addition to glyphosate to prevent the evolution of glyphosate resistance.

Resistance management conditions, as part of a license to grow Roundup Ready® cotton, currently encourage the use of as many different weed control options as possible, especially in high weed pressure situations. It also stipulates growers assess the incidence of escape weeds from Roundup Ready® herbicide applications and take whatever action is necessary to prevent seed set from weeds that have been exposed to glyphosate (Monsanto 2000). This prevention of seed set was shown to be important in Chapter 6, as both barnyardgrass and liverseed grass can produce large numbers of seed.

Conditions in the field may not require a fully integrated weed control program every year. Economic factors may also provide pressures to reduce the number of weed control practices used, especially expensive practices, such as hand-hoeing, or practices that impact on cotton development, such as residual herbicides. However, fields need to be closely monitored in order for effective action to be taken when the situation requires it. This is already a requirement of the Roundup Ready® crop management plan. It important that growers continue to utilise IWM practices in glyphosate-resistant cotton fields in order to continue to enjoy the benefits that glyphosate-resistant crops bring. It is also essential that the cotton industry continues to practice pro-active resistance management and that these issues are revisited as new herbicide resistance traits become available.

Appendices

Appendix 3.1

Table 1. Analysis of variance of in crop glyphosate use in fields with glyphosate-resistant technology and conventional fields across regions.

Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Technology	1	224.74	224.74	42.6	<0.001
Region	3	12.41	4.14	0.78	0.51
Technology x Region	3	9.04	3.01	0.57	0.64
Residual	72	379.81	5.28		
Total	79	625.99			

Table 2. Analysis of variance of glyphosate use in the crop rotation cycle in fields with glyphosate-resistant technology and conventional fields across regions.

Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Technology	1	27.11	27.11	34.17	<0.001
Region	3	2.15	0.72	0.9	0.44
Technology x Region	3	3.89	1.3	1.64	0.19
Residual	72	57.11	0.79		
Total	79	90.26			

Table 1. Analysis of variance of herbicides other than glyphosate in fields with glyphosate-resistant technology and conventional fields across regions.

Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Technology	1	13.9	13.9	7.29	0.009
Region	3	27.07	9.02	4.72	0.005
Technology x Region	3	4.89	1.63	0.85	0.47
Residual	72	137.75	1.91		
Total	79	183.61			

Table 2. Analysis of variance of herbicides other than glyphosate in fields with glyphosate-resistant technology and conventional fields in the Darling Downs region.

Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Technology	1	13.9	13.9	3.67	0.019
Residual	18	37.00	2.06		
Total	19	50.71			

Appendix 4.1

Table 1. Herbicides, herbicide rates and timings used in each treatment in systems experiment in the 2004-05 season

	RR Only		RR + IWM		RR + Res		RR + Grass		IWM Only	
Date	Herbicide	Rate g ai/ha	Herbicide	Rate g ai/ha	Herbicide	Rate g ai/ha	Herbicide	Rate g ai/ha	Herbicide	Rate g ai/ha
30/08/04			trifluralin	1104					trifluralin	1104
			diuron	1530					diuron	1530
			(cultivation to incorporate trifluralin/diuron)							
14/10/04	glyphosate[a]	450	glyphosate	450	glyphosate	450	glyphosate	450	glyphosate	450
(Cotton planted)			fluometuron	748	fluometuron	748			fluometuron	748
			prometryn	748	prometryn	748			prometryn	748
			pendimethalin	990	pendimethalin	990			pendimethalin	990
15/11/04	glyphosate	1035	glyphosate	1035	glyphosate	1035	glyphosate	1035		
26/11/04			Interrow cultivation						Interrow cultivation	
29/11/04	glyphosate	1035	glyphosate	1035	glyphosate	1035	glyphosate	1035		
10/01/05	glyphosate	1035	glyphosate	1035	glyphosate	1035	glyphosate	1035		
			prometryn	1080	prometryn	1080			prometryn	1080
11/01/05							haloxyfop	78	haloxyfop	78
03/05/05	(Cotton picked)									

[a] Glyphosate = *N*-phosphonomethyl glycine; trifluralin = 2,6-dintro-*N*,*N*-dipropyl-4-(trifluoromethyl)benzenamine; diuron = *N*'-(3,4-dichlorophenyl)-*N*,*N*-dimethylurea; fluometuron = *N*,*N*-dimethly-*N*'-[3-(trifluoromethyl)phenyl]urea; prometryn = *N*,*N*'-bis(1-methylethly)-6-(methylthio)-1,3,5-triazine-2,4-diamine; pendimethalin = *N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dintrobenzenamine; haloxyfop = (±)-2-[4-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid; diquat = 6,7-dihydrodipyrido[1,2-α:2'1'-*c*]pyrazinediium ion; paraquat = 1,1'-dimethyl-4,4'-bipyridinium ion.

Table 2. Herbicides, herbicide rates and timings used in each treatment in systems experiment in the 2005-06 season

	RR Only		RR + IWM		RR + Res		RR + Grass		IWM Only	
Date	Herbicide	Rate g ai/ha	Herbicide	Rate g ai/ha	Herbicide	Rate g ai/ha	Herbicide	Rate g ai/ha	Herbicide	Rate g ai/ha
16/09/05			trifluralin[a]	1104					trifluralin	1104
			diuron	1530					diuron	1530
			(cultivation and rainfall to incorporate trifluralin/diuron)							
19/09/05	Whole field accidentally sprayed with 1046.5 g ai/ha pendimethalin. The field was then rotary harrowed and irrigated so that it would be evenly spread throughout the profile to minimize its effect on the experiment.									
	paraquat	270	paraquat	270	paraquat	270	paraquat	270	paraquat	270
11/10/05	diquat	230	diquat	230	diquat	230	diquat	230	diquat	230
12/10/05			fluometuron	1800	fluometuron	1800			fluometuron	1800
(cotton planted)										
11/11/04	glyphosate	1035	glyphosate	1035	glyphosate	1035	glyphosate	1035	haloxyfop	78
9/12/04	glyphosate	1035	glyphosate	1035	glyphosate	1035	glyphosate	1035	haloxyfop	78
12/12/04			prometryn	1080	prometryn	1080			prometryn	1080
5/01/06							haloxyfop	78		
	(Cotton picked)									

[a] Glyphosate = *N*-phosphonomethyl glycine; trifluralin = 2,6-dintro-*N*,*N*-dipropyl-4-(trifluoromethyl)benzenamine; diuron = *N*’-(3,4-dichlorophenyl)-*N*,*N*-dimethylurea; fluometuron = *N*,*N*-dimethly-*N*’-[3-(trifluoromethyl)phenyl]urea; prometryn = *N*,*N*’-bis(1-methylethly)-6-(methylthio)-1,3,5-triazine-2,4-diamine; pendimethalin = *N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dintrobenzenamine; haloxyfop = (±)-2-[4-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid; diquat = 6,7-dihydrodipyrido[1,2-α:2’1’-*c*]pyrazinediium ion; paraquat = 1,1’-dimethyl-4,4’-bipyridinium ion.

Appendix 4.2

Table 1. Mean seedbank densities of barnyardgrass seeds under a range of weed management treatments as described in Appendix 4.1.

Treatment	**Date**	**Seedbank density (Seeds/m^2)**
RR Only	Sep 03	3378
RR + IWM		3251
RR + Res		1723
RR + Grass		3480
IWM Only		2224
Std error of mean		1158.1
LSD (p=0.05)		**NS**
RR Only	Sep 04	124
RR + IWM		15
RR + Res		27
RR + Grass		226
IWM Only		32
Std error of mean		74.8
LSD (p=0.05)		**NS**
RR Only	Aug 05	189
RR + IWM		15
RR + Res		5
RR + Grass		174
IWM Only		25
Std error of mean		86.3
LSD (p=0.05)		**NS**

Table 2. Latin square ANOVA table of seedbank densities of barnyardgrass seeds under the RR Only, RR + IWM, RR + Res, RR +Grass and IWM Only treatments as described in Appendix 4.1 for September 2003.

Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Row stratum	4	256308	64077	2.13	
Column stratum	4	157980	39495	1.31	
Row.Column stratum					
Treatment	4	129471	32368	1.08	0.411
Residual	12	360895	30075		
Total	24	904655			

Table 3. Latin square ANOVA table of seedbank densities of barnyardgrass seeds under the RR Only, RR + IWM, RR + Res, RR +Grass and IWM Only treatments as described in Appendix 4.1 for September 2004.

Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Row stratum	4	116596	29149	2.09	
Column stratum	4	39356	9839	0.7	
Row.Column stratum					
Treatment	4	162606	40652	2.91	0.068
Residual	12	167727	13977		
Total	24	486285			

Table 4. Latin square ANOVA table of seedbank densities of barnyardgrass seeds under the RR Only, RR + IWM, RR + Res, RR +Grass and IWM Only treatments as described in Appendix 4.1 for August 2005.

Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Row stratum	4	128593	32148	1.73	
Column stratum	4	41459	10365	0.56	
Row.Column stratum					
Treatment	4	168110	42028	2.26	0.123
Residual	12	223384	18615		
Total	24	561546			

Table 5. Mean seedbank densities of liverseed grass seeds under a range of weed management treatments as described in Appendix 4.1.

Treatment	Date	Seedbank Density (Seeds/m^2)
RR Only	Sep 03	85
RR + IWM		178
RR + Res		543
RR + Grass		34
IWM Only		153
Std error of mean		203.2
LSD (p = 0.05)		**NS**
RR Only	Sep 04	50
RR + IWM		52
RR + Res		274
RR + Grass		87
IWM Only		12
Std error of mean		157.6
LSD (p=0.05)		**NS**
RR Only	Aug 05	92
RR + IWM		7
RR + Res		52
RR + Grass		199
IWM Only		2
Std error of mean		109.7
LSD (p=0.05)		**NS**

Table 6. Latin square ANOVA table of seedbank densities of liverseed grass seeds under the RR Only, RR + IWM, RR + Res, RR +Grass and IWM Only treatments as described in Appendix 4.1 for September 2003.

Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Row stratum	4	300879	75220	0.73	
Column stratum	4	552332	138083	1.34	
Row.Column stratum					
Treatment	4	806667	201667	1.95	0.166
Residual	12	1239108	103259		
Total	24	2898985			

Table 7. Latin square ANOVA table of seedbank densities of liverseed grass seeds under the RR Only, RR + IWM, RR + Res, RR +Grass and IWM Only treatments as described in Appendix 4.1 for September 2004.

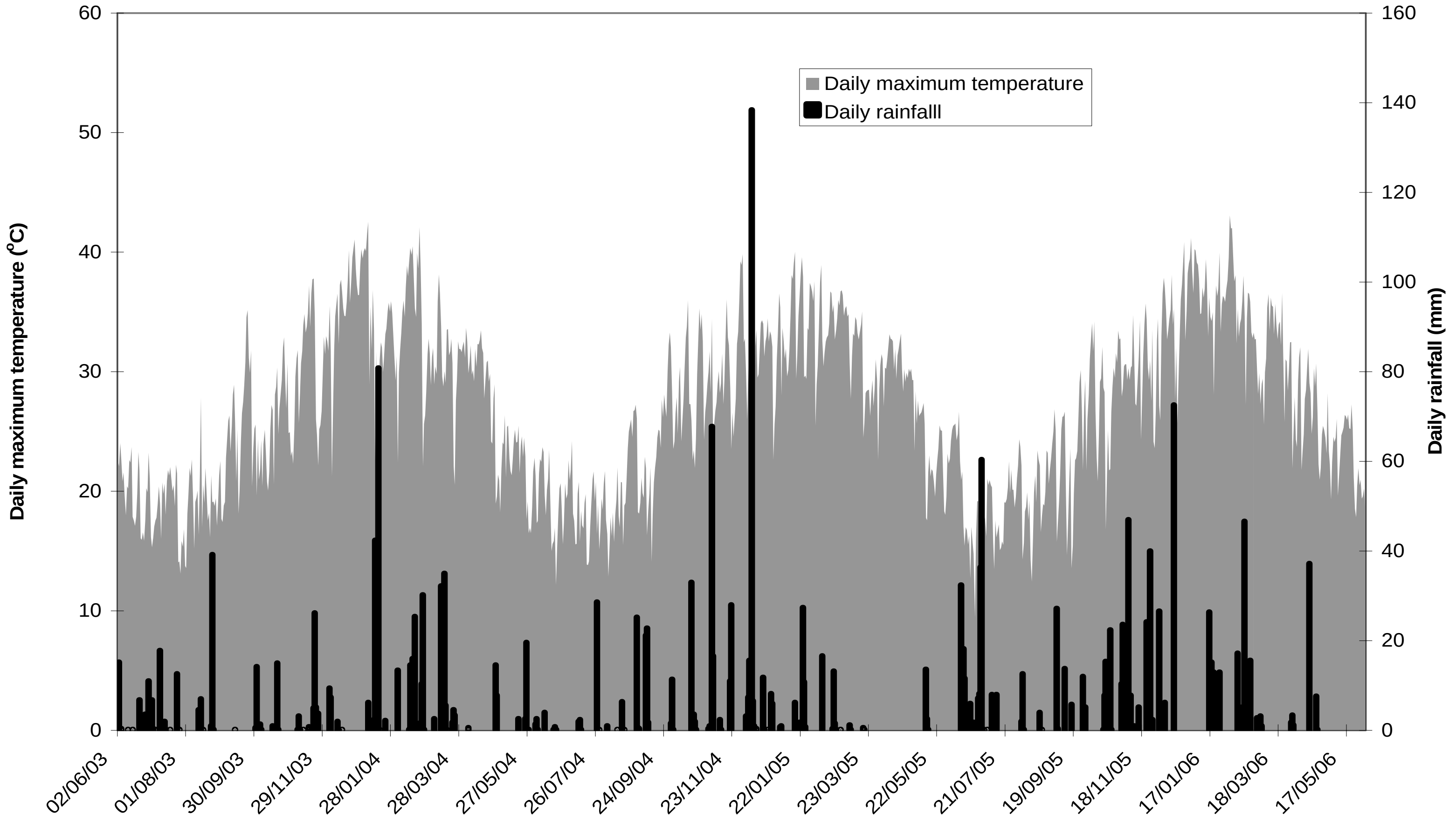
Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Row stratum	4	219748	54937	0.89	
Column stratum	4	395192	98798	1.59	
Row.Column stratum					
Treatment	4	213193	53298	0.86	0.516
Residual	12	744770	62064		
Total	24	1572902			

Table 8. Latin square ANOVA table of seedbank densities of liverseed grass seeds under the RR Only, RR + IWM, RR + Res, RR +Grass and IWM Only treatments as described in Appendix 4.1 for August 2005.

Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Row stratum	4	256308	64077	2.13	
Column stratum	4	157980	39495	1.31	
Row.Column stratum					
Treatment	4	129471	32368	1.08	0.411
Residual	12	360895	30075		
Total	24	904655			

Appendix 5.1

Figure 1. Daily rainfall and maximum temperature at the Australian Cotton Research Institue, Narrabri from June 2003 to June 2006.



Appendix 5.2

Table 1. ANOVA table for barnyardgrass above-ground biomass per plant in season 2003-04 (square root transformed)

Source	DF	Sum of squares	Mean square	Variance ratio	F. pr.
Density	3	634.053	211.351	38.2	<.001
Residual	12	66.397	5.533		
Total	15	700.45			

Table 2. Factorial ANOVA table for barnyardgrass above-ground biomass per plant in season 2004-05 (square root transformed).

Source	DF	Sum of squares.	Mean square	Variance ratio	F pr.
Planting date	1	5708.926	5708.926	604.73	<.001
Density	4	2627.381	656.845	69.58	<.001
Planting date x Density	4	362.457	90.614	9.6	<.001
Residual	110	1038.449	9.44		
Total	119	9737.213			

Table 3. ANOVA table for barnyardgrass above-ground biomass per plant for the first planting in 2004-05 (square root transformed).

Source	DF	Sum of squares.	Mean square	Variance ratio	F pr.
Density	5	3373.831	674.766	88.49	<.001
Residual	66	503.284	7.626		
Total	71	3877.115			

Table 4. ANOVA table for barnyardgrass above-ground biomass per plant for the second planting in 2004-05 (square root transformed).

Source	DF	Sum of squares.	Mean square	Variance ratio	F pr.
Density	5	853.221	170.644	18.16	<.001
Residual	66	620.159	9.396		
Total	71	1473.381			

Table 5. ANOVA table for liverseed grass above-ground biomass per plant in season 2003-04 (square root transformed).

Source	DF	Sum of squares	Mean square	Variance ratio	F. pr.
Density	3	22.86	7.62	2.71	0.092
Residual	12	33.764	2.814		
Total	15	56.624			

Table 6. Factorial ANOVA for liverseed grass above-ground biomass per plant in season 2004-05 (square root transformed).

Source	DF	Sum of squares	Mean square	Variance ratio	F. pr.
Planting date	1	4347704	4347704	412.93	<0.001
Density	5	2264132	452826	43.01	<0.001
Planting date x Density	5	1996275	399255	37.92	<0.001
Residual	180	1895215	10529		
Total	191	10503327			

Appendix 5.3

Table 1. ANOVA table for barnyardgrass seed production per plant in season 2003-04 (square root transformed).

Source	DF	Sum of squares	Mean square	Variance ratio	F. pr.
Density	3	17111.7	5703.9	45.16	<.001
Residual	12	1515.6	126.3		
Total	15	18627.3			

Table 2. Factorial ANOVA table for barnyardgrass seed production per plant in season 2004-05 (square root transformed).

Source	DF	Sum of squares	Mean square	Variance ratio	F. pr.
Planting date	1	49189.8	49189.8	69.05	<.001
Density	4	32660.5	8165.1	11.46	<.001
Planting date x Density	4	5950.4	1487.6	2.09	0.107
Residual	30	21370.6	712.4		
Total	39	109171.3			

Table 3. ANOVA table for barnyardgrass seed production per plant for the first planting in 2004-05 (square root transformed).

Source	DF	Sum of squares	Mean square	Variance ratio	F. pr.
Density	5	4.49E+04	8.97E+03	30.13	<.001
Residual	18	5.36E+03	2.98E+02		
Total	23	5.02E+04			

Table 4. ANOVA table for barnyardgrass seed production per plant for the second planting in 2004-05 (square root transformed).

Source	DF	Sum of squares	Mean square	Variance ratio	F. pr.
Density	5	9.74E+03	1.95E+03	2.16	0.104
Residual	18	1.62E+04	9.00E+02		
Total	23	2.59E+04			

Table 5. ANOVA table for liverseed grass seed production per plant in season 2003-04 (LOG_{10} transformed).

Source	DF	Sum of squares	Mean square	Variance ratio	F. pr.
Density	3	2.89315	0.96438	54.23	<.001
Residual	12	0.21341	0.01778		
Total	15	3.10657			

Table 6. Factorial ANOVA for liverseed grass seed production per plant in season 2004-05 (square root transformed).

Source	DF	Sum of squares	Mean square	Variance ratio	F. pr.
Planting date	1	192618.6	192618.6	1126.67	<.001
Density	5	57129.8	11426	66.83	<.001
Planting date x Density	5	18881.3	3776.3	22.09	<.001
Residual	180	30773.4	171		
Total	191	299403.1			

Appendix 5.4

Table 1. Analysis of variance for barnyardgrass seed production per meter in season 2003-04.

Source	DF	Sum of squares	Mean square	Variance ratio	F. pr.
Density	3	1.43E+09	4.77E+08	1.88	0.186
Residual	12	3.04E+09	2.53E+08		
Total	15	4.47E+09			

Table 2. Factorial ANOVA table for barnyardgrass seed production per meter in season 2004-05 for densities of 0.5, 1, 2, 5, and 10 plants per meter.

Source	DF	Sum of squares	Mean square	Variance ratio	F. pr.
Planting date	1	4.33E+09	4.33E+09	73.46	<.001
Density	4	1.16E+09	2.91E+08	4.93	0.004
Planting date x Density	4	3.84E+08	9.60E+07	1.63	0.193
Residual	30	1.770E+09	5.899E+07		
Total	39	7.65E+09			

Table 3. ANOVA table for barnyardgrass seed production per meter for the 1st planting in 2004-05.

Source	DF	Sum of squares	Mean square	Variance ratio	F. pr.
Density	5	1.90E+09	3.80E+08	7.13	<.001
Residual	18	9.58E+08	5.32E+07		
Total	23	2.86E+09			

Table 4. ANOVA table for barnyardgrass above ground biomass per meter for the 2nd planting in 2004-05.

Source	DF	Sum of squares	Mean square	Variance ratio	F. pr.
Density	5	8289	1658	0.66	0.656
Residual	18	4.50E+04	2.50E+03		
Total	23	5.33E+04			

Table 5. ANOVA table for liverseed grass seed production per meter in season 2003-04.

Source	DF	Sum of squares	Mean square	Variance ratio	F. pr.
Density	3	9.07E+08	3.02E+08	1.08	0.395
Residual	12	3.37E+09	2.81E+08		
Total	15	4.27E+09			

Table 6. Factorial ANOVA for liverseed grass seed production per meter in season 2004-05 (square root transformed).

Source	DF	Sum of squares	Mean square	Variance ratio	F. pr.
Planting date	1	741619.4	741619.4	1864.8	<.001
Density	5	170002	34000.4	85.49	<.001
Planting date x Density	5	113260.6	22652.1	56.96	<.001
Residual	180	71584.7	397.7		
Total	191	1096466.8			

Appendix 5.5

Table 1. Parameter estimates (mean ± SE),and correlation coefficients for seed production per plant in relation to above ground biomass per plant in figure 5. Data was fitted to a linear regression model $y = ax + y0$.

		a		***y0***			
		Mean	**SE**	**Mean**	**SE**	**R^2**	**F pr.**
Barnyard grass							
Season 2004-05	1st Plant	32.24	3.97	-1032.63	232.90	0.75	<0.001
	2nd Plant	49.79	4.28	-422.28	684.86	0.62	<0.001
Season 2003-04		67.90	7.08	852.16	344.64	0.63	<0.001
Liverseed grass							
Season 2004-05	1st Plant	18.27	1.25	1444.45	479.98	0.90	<0.001
	2nd Plant	44.46	0.39	90.94	47.61	0.84	<0.001
Season 2003-04		44.32	1.65	744.88	252.18	0.73	<0.001

Table 2. Parameter estimates (mean ± SE), correlation coefficients, and rectangular hyperbolic regression significance probabilities for barnyardgrass and liverseed grass.

		Barnyardgrass						Liverseed grass					
		a		***b***				***a***		***b***			
		Mean	**SE**	**Mean**	**SE**	**R^2**	**F pr.**	**Mean**	**SE**	**Mean**	**SE**	**R^2**	**F pr.**
Season 2004-05													
1st Plant	Biomass/plant	1101.23	208.88	1.61	0.75	0.92	0.002	725.17	83.99	2.29	0.74	0.96	<.001
	Seeds/plant	41377.24	7209.35	1.04	0.39	0.97	<.001	13703.46	1500.48	3.84	1.39	0.94	0.001
	Seeds/meter	40137.86	2474.19	0.93	0.24	0.93	0.002	65395.79	6933.29	7.08	2.09	0.95	<.001
2nd Plant	Biomass/plant	243.67	73.00	0.97	0.61	0.92	0.002	59.88	9.96	0.46	0.13	0.99	<.001
	Seeds/plant	9531.86	3316.96	1.15	0.89	0.87	0.007	2194.57	930.07	0.84	0.71	0.88	0.005
	Seeds/meter	13014.86	1760.90	1.73	0.86	0.81	0.014	1969.33	458.98	1.35	1.22	0.47	0.132
Season 2003-04													
	Biomass/plant	3622.19	17256.21	0.31	1.54	0.97	0.017	89.78	27.84	9.37	6.09	0.93	0.037
	Seeds/plant	35156.52	16414.73	1.54	0.90	0.99	0.004	10275.65	3381.28	3.58	1.79	0.98	0.009
	Seeds/meter	-	-	-	-	-	-	30307.73	3047.71	1.60	1.23	0.58	0.241

a, maximum seed production; *b*, change in seed production.

Appendix 6.1

Table 1. Factorial ANOVA for dose-mortality response of barnyardgrass and liverseed grass treated with glyphosate at the two-leaf growth stage in 2003.

Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Dose rate	2	973.8	486.9	3.8	0.03
Species	1	55.2	55.2	0.43	0.515
Dose rate x Species	2	421.3	210.6	1.64	0.205
Residual	42	5378.1	128.1		
Total	47	6828.4			

Table 2. Factorial ANOVA for dose-mortality response of barnyardgrass and liverseed grass treated with glyphosate at the four-leaf growth stage in 2003.

Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Dose rate	2	89.38	44.69	1.53	0.228
Species	1	5.59	5.59	0.19	0.664
Dose rate x Species	2	108.32	54.16	1.86	0.169
Residual	42	1224.81	29.16		
Total	47	1428.09			

Table 3. Factorial ANOVA for dose-mortality response of barnyardgrass and liverseed grass treated with glyphosate at the six-leaf growth stage in 2003.

Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Dose rate	2	416.4	208.2	1.43	0.25
Species	1	1051.1	1051.1	7.24	0.01
Dose rate x Species	2	305.2	152.6	1.05	0.359
Residual	42	6097.4	145.2		
Total	47	7870.2			

Table 4. Factorial ANOVA for dose-mortality response of barnyardgrass and liverseed grass treated with glyphosate at the two-leaf growth stage in 2004.

Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Dose rate	3	4997.82	1665.94	20.05	<.001
Species	1	878.6	878.6	10.57	0.002
Dose rate x Species	3	1077.19	359.06	4.32	0.007
Residual	88	7312.12	83.09		
Total	95	14265.74			

Table 5. Factorial ANOVA for dose-mortality response of barnyardgrass and liverseed grass treated with glyphosate at the six-leaf growth stage in 2004.

Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Dose rate	3	2395.72	798.57	12.09	<.001
Species	1	230.29	230.29	3.49	0.065
Dose rate x Species	3	674.63	224.88	3.41	0.021
Residual	88	5810.83	66.03		
Total	95	9111.47			

Table 6. Factorial ANOVA for dose-mortality response of barnyardgrass and liverseed grass treated with glyphosate at the two-leaf growth stage in 2005.

Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Dose rate	4	17762.18	4440.55	52.26	<.001
Species	1	1631.07	1631.07	19.19	<.001
Dose rate x Species	4	2553.81	638.45	7.51	<.001
Residual	80	6798.13	84.98		
Total	89	28745.19			

Table 7. Factorial ANOVA for dose-mortality response of barnyardgrass and liverseed grass treated with glyphosate at the four-leaf growth stage in 2005.

Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Dose rate	4	82518.1	20629.5	142.55	<.001
Species	1	8.7	8.7	0.06	0.807
Dose rate x Species	4	580.2	145.1	1	0.411
Residual	80	11577.5	144.7		
Total	89	94684.6			

Appendix 6.2

Table 1. Factorial ANOVA for barnyardgrass dose-mortality response to increasing rates of glyphosate in season 2003-04 (square root transformed).

Source	DF	Sum of squares	Mean square	Variance ratio.	F pr.
Dose rate	2	523.15	261.63	3.52	0.04
Growth stage	2	484.66	242.33	3.26	0.05
Dose rate x Growth stage	4	131.55	32.89	0.44	0.777
Residual	27	2007.75	74.36		
Total	35	3147.72			

Table 2. Factorial ANOVA for barnyardgrass dose-mortality response to increasing rates of glyphosate in season 2004-05 (square root transformed).

Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Dose rate	3	27.62	9.21	11.09	<.001
Growth stage	1	2.50	2.49	3	0.009
Dose rate x Growth stage	3	1.29	0.43	0.52	0.673
Residual	24	19.91	0.82		
Total	31	51.31			

Table 3. Factorial ANOVA for barnyardgrass dose-mortality response to increasing rates of glyphosate in season 2005-06 (square root transformed).

Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Dose rate	4	206.02	51.50	108.19	<.001
Growth stage	1	40.46	40.46	85	<.001
Dose rate x Growth stage	4	24.57	6.14	12.9	<.001
Residual	20	9.52	0.47		
Total	29	280.57			

Table 4. Factorial ANOVA for liverseed grass dose-mortality response to increasing rates of glyphosate in season 2003-04 (square root transformed).

Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Dose rate	2	241.59	120.79	6.27	0.006
Growth stage	2	280.01	140	7.26	0.003
Dose rate x Growth stage	4	260.8	65.2	3.38	0.023
Residual	27	520.49	19.28		
Total	35	1302.88			

Table 5. Factorial ANOVA for liverseed grass dose-mortality response to increasing rates of glyphosate in season 2004-05 (square root transformed).

Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Dose rate	3	86.88	28.96	18.61	<.001
Growth stage	1	13.78	13.78	8.86	0.007
Dose rate x Growth stage	3	3.35	1.12	0.72	0.552
Residual	24	37.35	1.56		
Total	31	141.36			

Table 6. Factorial ANOVA for liverseed grass dose-mortality response to increasing rates of glyphosate in season 2005-06 (square root transformed).

Source	DF	Sum of squares	Mean square	Variance ratio	F pr.
Dose rate	4	295.13	73.35	96.8	<.001
Growth stage	1	14.03	14.03	18.41	<.001
Dose rate x Growth stage	4	20.71	5.17	6.79	0.001
Residual	20	15.24	0.76		
Total	29	345.11			

Appendix 6.3

Table 1. Parameter estimates (mean ± SE), and correlation coefficients for barnyardgrass dose-mortality response to increasing rates of glyphosate. Data was fitted to an exponential decay regression model $y = a * \exp(-b*x)$.

Season	Growth Stage	*a*		*b*			
		Mean	SE	Mean	SE	R^2	F pr.
2003	2 Leaf	99.693	8.492	0.005	0.002	0.977	0.012
	4 Leaf	99.997	0.753	0.008	0.000	1.000	<0.001
	6 Leaf	99.754	7.746	0.006	0.001	0.981	0.010
2004	2 Leaf	99.968	1.759	0.013	0.001	0.999	<0.001
	6 Leaf	99.995	0.713	0.016	0.001	1.000	<0.001
2005	2 Leaf	99.478	4.3615	0.0158	0.0018	0.9898	<0.0001
	6 Leaf	100	6.459	0.0039	0.0006	0.9782	0.0002

a, maximum survivors with no glyphosate applied; *b*, survivor reduction coefficient under glyphosate application.

Table 2. Parameter estimates (mean ± SE), and correlation coefficients for liverseed grass dose-mortality response to increasing rates of glyphosate. Data was fitted to an exponential decay regression model $y = a * \exp(-b*x)$.

Season	Growth Stage	*a*		*b*			
		Mean	SE	Mean	SE	R^2	F pr.
2003	2 Leaf	99.994	2.950	0.006	0.001	0.997	0.001
	4 Leaf	99.999	3.346	0.012	0.006	0.997	0.002
	6 Leaf	100.000	1.007	0.030	0.309	1.000	0.000
2004	2 Leaf	99.748	2.003	0.007	0.000	0.998	<0.001
	6 Leaf	100.037	0.702	0.010	0.000	1.000	<0.001
2005	2 Leaf	99.8118	3.3503	0.008	0.0006	0.9939	<0.0001
	6 Leaf	100	4.1644	0.0039	0.0004	0.99	<0.0001

a, maximum survivors with no glyphosate applied; *b*, survivor reduction coefficient under glyphosate application.

Table 1. Default parameter settings using in resistance model simulations in Chapter 8. Values are set to these values unless otherwise stated in the text.

Parameter	Description	Default
Field	Field size	50 ha
Sbank_i	Initial seedbank density per meter	750
GlyDose1	Rate of glyphosate used in pre-plant knockdown	450 g a.i./ha
GlyDose2	1st Roundup Ready® herbicide spray	1035 g a.i./ha
GlyDose3	2nd Roundup Ready® herbicide spray	1035 g a.i./ha
GlyDose4	3rd Roundup Ready® herbicide spray	1035 g a.i./ha
$P(S_{RRG})$	Probability of resistant alleles surviving glyphosate application	1
$P(S_{RrG})$	Probability of heterozygous alleles surviving glyphosate application	0.8
$P(S_H)$	Probability of weeds surviving haloxyfop application	0.5 – 0.15
$P(S_P)$	Probability of weeds surviving Prometryn lay-by application	0.3 – 0.4
$P(S_T)$	Probability of weeds surviving tillage (percent area covered by inter-row cultivator)	0.7
Surv_{MAX}	Maximum survival of weeds sprayed with glyphosate	99.7
Seed_{MAX}	Maximum seed production per meter – Barnyard grass	13015
	- Liverseed grass	1969
ΔSeed_A	Change in seed production per meter – Barnyard grass	1.73
	- Liverseed grass	1.35
Fp_R	Fitness penalty of homozygous resistant alleles	0.3
Fp_{Rr}	Fitness penalty of heterozygous alleles	0.15
%Self	Percent self fertilising – Barnyard grass	0.85
	- Liverseed grass	0.6

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