FINAL REPORT

For Public Release

Part 1 - Summary Details

CRDC ID: DAN1901

Project Title: Integrated weed management options for weed control in cotton farming systems

Project Start Date: 01/07/2018 **Project Completion Date:** 30/09/2019

Research Program: 1 Farmers

Part 2 – Contact Details

Administrator: Amanda Waterman
Organisation: NSW Department of Primary Industries
Postal Address: Locked bag Orange, NSW 2800
Ph: 0263913545 Fax: E-mail: contract.admin@dpi.nsw.gov.au

Principal Researcher: Eric Koetz
Organisation: NSW Department of Primary Industries
Postal Address: WWAI, PMB, Pine Gully Road, Wagga Wagga NSW 2560
Ph: 0269381954 Fax: E-mail: eric.koetz@dpi.nsw.gov.au

Supervisor: Dr Sandra McDougall
Organisation: NSW Department of Primary Industries
Postal Address: PMB Yanco NSW 2703
Ph: 0269512728 Fax: E-mail: Sandra.mcdougall@dpi.nsw.gov.au

Researcher 2: Dr Asad Asaduzzaman
Organisation: NSW Department of Primary Industries
Postal Address: WWAI, PMB Pine Gully Road, Wagga Wagga, NSW 2650
Ph: 0269381833 Fax: E-mail: asad.asaduzzaman@dpi.nsw.gov.au

Signature of Research Provider Representative:

Date submitted: ______________________

Part 3 – Final Report

Background

Outline the background to the project.

This 15 month project built on the previous CRDC project **DAN1402** ***Hard to control weeds in the northern cotton farming system*** and many years of weeds work supported by CRDC and GRDC. The aim was to value add to the earlier work, while providing strategic information to growers in support of Integrated Weed Management (IWM) and the cotton industries' best practice guidelines, MyBMP, and improving information on hard to control weeds and herbicide resistant weeds. The project aimed to evaluate pre- and post-emergent chemistry, residual and layby herbicide applications and integrate chemical and non-chemical tactics. The project included a three month variation from the original 12 month project to allow glasshouse and trial data to be finalised and compiled into technical reports. A series of demonstration trials were established with test strips evaluating application timings and strategies for layby and residual herbicides and used as extension opportunities with grower groups and industry events.

In the past 12 years, a considerable RD& E effort at preventing and combating herbicide resistance in northern NSW and SE Qld has occurred. Despite this, the resistance issues in grains/cotton production systems are increasing. There are many research gaps yet to be answered. The increasing number of resistant weed species and the various methods to control these weeds needs to be thoroughly investigated. The potential to incorporate “area-wide management” as part of an integrated approach to weed management was investigated. Additional support from CRDC and NSW DPI would complement previous and current industry efforts and meet some of these research gaps that are crucial to preserving farming systems in Australia from the threat of resistant weeds.

Industry wide adoption of IWM principles and herbicide resistant management strategies remains a critical goal for both the cotton and grains industries. Despite industry surveys indicating a high level of awareness of herbicide resistance issues (@22% CRDC Grower survey 2017), cotton and grains growers continue to rely heavily on herbicide (particularly glyphosate) applications as the dominant method for weed control. This project will promote alternative tactics to improve weed and survivor control in glyphosate tolerant cotton systems.

Objectives

List the project objectives and the extent to which these have been achieved, with reference to the Milestones and Performance indicators.

Milestone .1 What are the current industry practice and emerging issues for weed management

Expand on grower case studies, deliver Patch Management updates and include questions in CCA grower survey

Dry conditions across the cotton valleys made sourcing patches of weeds difficult. No farm walks targeting patch management were conducted. Content around managing small weed numbers and targeting herbicide application was included in CottonInfo videos, cotton e-news and WEEDsmart blogs and ask an expert.

Milestone 2. What are the knowledge gaps in ecology, biology of the emerging weeds identified

2.1. Biology and ecology studies in glasshouse and field.

Button grass (*Dactyloctenium radulans*) is a native summer grass species common in cotton growing regions in Australia. Button grass germinates across a wide range of day/night temperature regimes. The seed responds to light stimulus and as such readily germinates from the soil surface. The seeds germinate in a pH range from 4–10 but higher numbers emerged from the more alkaline conditions.

Bladder ketmia (*Hibiscus trionum var. trionum*) is a highly competitive weed with cotton and biology studies showed significant reductions in cotton root biomass and leaf area and delays in appearance of first square.

A cooperative approach to management of rosin weed (*Cressa cretica*) with a local agronomist at Forbes in the Lachlan valley has been placed on hold awaiting suitable seasonal conditions. Glyphosate does not control the weed and residual options are limited as these fields are in cotton farming systems. An opportunity exists to conduct a replicated spray grid experiment if seasonal conditions improve.

Four fact sheets (Feathertop Rhodes grass, Windmill grass, Fleabane and Sowthistle) are either awaiting formatting for publication or in draft version awaiting approval for publication via industry channels.

Milestone 3. Are there alternative non-glyphosate tactics, chemical (residual, layby) and non-chemical IWM tactics?

3.1. Fact sheets, new or existing chemistry for pre or post emergent application to reduce glyphosate use.

The IWM section of the Cotton Pest Management Guide has been updated to include new registrations. As part of the messaging around introducing alternative herbicides in place of glyphosate, the Bayer Roundup Ready Plus system has added new herbicides and the uptake in the first season has been an impressive 25%. Demonstration trial results from IREC and Wee Waa were extended to growers and industry in newsletters and industry publications.

3.2 Select sites for IWM demonstrations including residuals at 3 locations.

Two demonstration sites were established, one in Wee Waa and the other at the IREC research farm at Whitton. Industry field days were held at both sites with attendance of approximately 180 people across both days. Individual visits by industry agronomists and a field walk were conducted at the Whitton site. Where pre-emergent treatments were applied, weed pressure was significantly lower than control or glyphosate alone. A presentation about the trial was included as part of the CSD farm tour and field day. Articles and extension material has appeared in e-news, a CottonInfo video, IREC farmer newsletter, WEEDsmart blog and ask an expert series and REO grower newsletters.

Milestone 4. What are the major regional weeds emerging in cotton farming systems and the current herbicide resistance status in the region.

4.1 Conduct regional weed survey throughout cotton farming systems

Due to very dry conditions across much of the cotton valleys in 2018–19 there was limited opportunity to gather weed seeds. All samples collected have been screened and significant levels of glyphosate resistance are present, especially in grass species. Feedback from growers on an informal basis at field days and farm walks supported low weed pressure throughout the season. A summary of the resistance status of weeds collected in the previous surveys is included in results.

Milestone 5. What role does pupae busting play in an IWM system to control weeds?

5.1. Impacts of pupae busting on IWM

The pupae busting experiment at ACRI was modified with the design including three additional lower input subplot treatments, adding: T4 (at-planting residual herbicides only [no glyphosate]); T5 (at-planting and in-crop residual herbicides only [no glyphosate]); and T6 (glyphosate only). Earlier treatments had not resulted in different weed burdens and the decision was made to expand the range of treatments with an emphasis on differing levels of weed control. The cultivation treatments late in the crop are supporting lower weed numbers as modelled in the Herbicide Resistance Management Strategy (HRMS).

The impact of the change in design, with a relaxation in the very high level of weed control previously imposed, was evident in the first season, with increased weed pressure and a trend towards differences between treatments, with lower weed pressure apparent on the centre busting treatment, although the trend was not statistically significant.

Milestone 6. CottonInfo Technical Lead for Weed Management, role of industry specialist.

6.1. 2. Industry specialist.

Weed related communication articles and content has been developed and appeared in Spotlight, The Australian Cotton Grower and WEEDsmart. Eric has established regular communication with the new Cotton research Agronomist at Yanco, Hayden Petty and the new Cotton Pathologist, Tim Green. A presentation for weed management was included in the Cotton 101 session at the cotton collective in Griffith. Eric has participated in two video and three phone meetings with the Herbicide Technical Panel and the Xtend Advisory group. Industry agronomists and growers have contacted Eric via phone to discuss emerging weed issues and possible strategies for control (annual ryegrass in south).

6.3 Technical area and extension lead.

Two cotton e-news communications were delivered this season and presentations at CGA field days and grower walks focussed on reducing the weed seed bank with a diverse approach to weed control. The CottonInfo video series has been updated with two new weed videos.

Extension material has been developed for CottonInfo REO newsletters, WEEDsmart, Spotlight, grower newsletters and The Australian Cottongrower. The CottonInfo team has developed a strategy for delivering on weed issues in the coming season and a presentation was presented at the AACS conference in Armidale in October.

6.4 Technical area extension lead.

A series of weed fact sheets are being produced with the assistance of the CRDC Communication manager and e-news content has been delivered this season. I have attended a CottonInfo team meeting in Griffith for AOP planning and setting the work program for 2019–20 in the weed management section of the plan. Fortnightly phone meetings are held with the team to update on current and future activities. The CottonInfo AOP is updated and reviewed in *Asana* as part of the M and E for the role.

6.5 Technical area extension lead

Working with Kieran O'Keefe, Southern CottonInfo officer to ensure the CottonInfo weed management program is in place for 2019–20 season.

6.6 Technical area extension lead

As part of our fortnightly phone meetings the CottonInfo team is kept up to date on weed issues and content. The weed management section of *Asana* is kept relevant and updated.

6.7 Technical area extension lead.

The weed management program within the CottonInfo network is adjusted as required and consultation with REO partner is undertaken on a regular basis.

6.8 Technical area extension lead

At CottonInfo team meetings I set the weed management agenda and lead discussions to develop plans for the season in consultation with the REOs and CottonInfo Program manager.

6.9 Best practice reviewer

The myBMP module is up to date and is in the process of being re-reviewed and will be updated if required. The Cotton Pest Management Guide and Australian Cotton Production Manual have had the chapters on weed management updated and refreshed. The Herbicide Technical Panel has had two face to face and two video meetings and a recommendation has been presented to the TIMS committee on the Xtend cotton system. Regular communication is occurring with members of the Herbicide Technical Panel on this and other issues. The Xtend Advisory Group has had one video meeting and one face to face meeting in an effort to establish a good robust stewardship package for Xtend cotton.

Methods

Detail the methodology and justify the methodology used. Include any discoveries in methods that may benefit other related research.

Detailed methodology and results are contained in the next section.

Results

Detail and discuss the results for each objective including the statistical analysis of results.

Detailed below are the results from glasshouse and field experiments conducted during the project. The three month extension allowed this program of work to be analysed and the reports finalized.

Experimental reports

Cotton seedlings response to simulated drift of 2, 4-D

Key findings

- Cotton plant can be injured by low doses of 2, 4-D and symptoms can be visible after 7 or more days of herbicide application.
- Leaves are more sensitive than shoot or other plant parts
- Low dose may not result in any visable symptoms but the plant can still lose biomass production

Rationale

Herbicide drift, especially auxin herbicide, has resulted in significant losses to cotton crops. Relating damage, injury intensity and symptoms on cotton plants at various phenological stages and growing conditions, as well as weather conditions (eg. inversions) during application and the amount of active ingredient reaching the recipient cotton plant will help with understanding of the impact of spray drift. Very little information is available to describe cotton response to different doses of 2, 4-D on cotton seedlings.

Objective

The objectives of this research was to determine cotton response to different doses of 2, 4 -D at four leaf stage of cotton growth in glasshouse conditions.

Approaches

The injury rating scale was constructed by observing and categorising injury to test plants exposed to reduced rates of 2, 4 -D (Table 1). The visual estimates of plant injury were recorded at 1, 7, 14 and 21 days after treatment (DAT).

Table 1: Injury rating of observed leaves.

Rating	Observed symptomology
1–3	Slight ripples in leaf margin creating ''draw-string'' effect. Isolated wart-like growths may also be observed on the upper epidermis of the leaf.
4–6	Ripples are more pronounced and present on at least 50% of leaf margin. Wart-like growths on leaf are common.
7–10	Leaves appear stiff and brittle. Stiff areas may also display chlorosis. Necrosis begins to appear around leaf margin which is uneven and gnarled throughout its perimeter.
11–15	Chlorosis is more obvious. Necrosis becomes more prominent and affects no more than 10% of the total new leaf area.
16–20	Necrosis is the dominant factor affecting up to 10–20% of the young leaves. Epinasty is severe around the entire leaf margin.
21–30	Up to 20–30% of the leaf tissue is necrotic, with chlorosis apparent through much of the leaf perimeter.
31-40	The leaf is very disfigured and chlorotic. Necrosis is evident on up to 30–40% of the leaf.
41-100	Necrosis becomes the primary indicator of plant injury (>40%). Although epinasty is extreme throughout the leaves, chlorosis and necrosis coverage is dominant

Results

Cotton Injury and Symptoms:

The majority of the injury recorded from cotton was observed on leaves. There was no injury recorded after 1 day of 2, 4-D application (DAT). The injury appeared after 7 days of 2, 4-D application and increased with herbicide doses (Figures 1 & 2). The average injury value at 2.5% 2, 4-D for leaves at 7, 14 and 21 DAT were 3, 6 and 17.5 (Please see Table 1) respectively. These injury values were greatest at 5% of 2, 4-D.

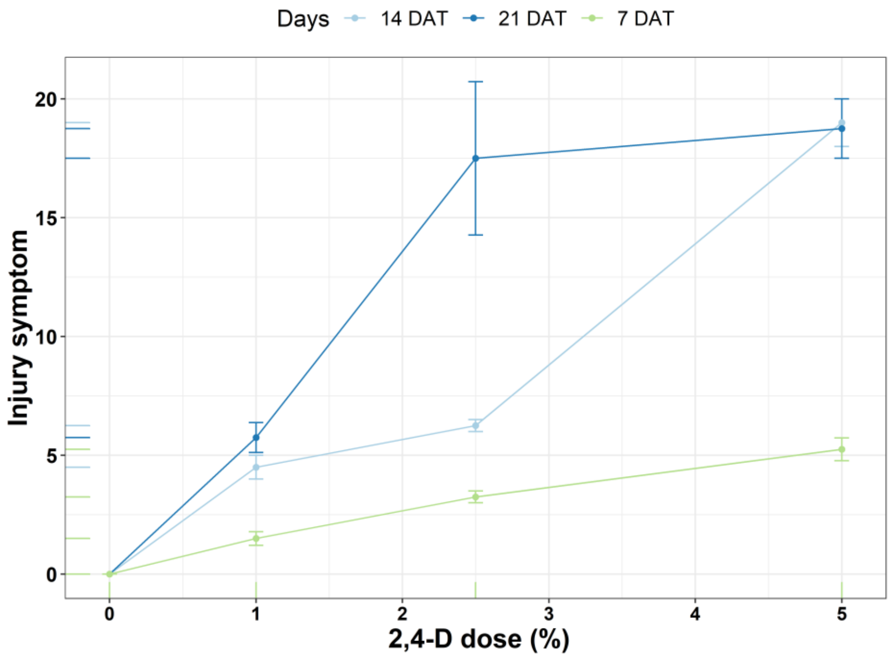


Figure 1 Cotton leaf injury due to different doses of 2, 4-D herbicide. The symptoms were visible after 7, 14 and 21 DAT. (See Table 1 for injury level).

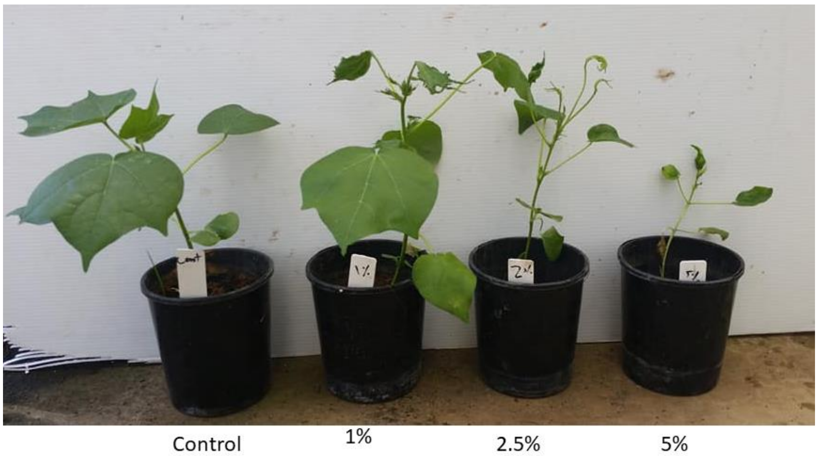


Figure 2 Cotton leaf injury due to different doses of 2, 4-D. The symptoms were visible after 14 DAT. The injury value 0, 10, 15 and 20 are for herbicide dose 0, 1%, 2.5% and 5% respectively (See Table 1).

Plant height (cm)

Plant height was initially stimulated due to very low rate (1%) of 2, 4-D, after that declined as herbicide rate was increased (Figure 3).

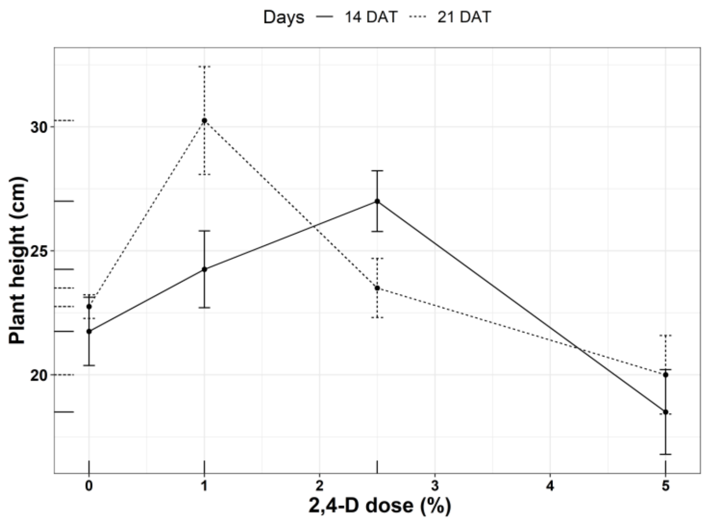


Figure 3 The plant height of cotton seedlings after exposure to 4 low rates of 2, 4-D herbicide.

Plant biomass at 21 DAT

Above-ground biomass was significantly affected by herbicide dose (Table 2). The maximum biomass was harvested in the control treatment.

Table 2 Above-ground biomass of cotton at 21 days after exposure to different rates of 2, 4-D.

Dose (%)	**Biomass (g)**	**SE(standard error)**
0	9.7	0.27
1	9.7	0.34
2.5	8.7	1.0
5	8	1.0

Herbicide hormesis, paraquat low dose implications in Tall fleabane (*C. sumatrensis*)

Key findings

This is the first report of paraquat resistance of *C. sumatrensis* in Australia where populations were sourced from cotton cropping systems. The results also disclose that both tested populations showed a hormetic growth increase at two lower doses (62.5 and 125 g a. i/ha) of paraquat, leading to fitness enhancements of plant height, above-ground biomass and buds plant^{-1} over untreated plants. The hormetic effects resulted in higher fitness at the vegetative stage with an increase of 52%; and 23% (for 62.5 a.i ha^{-1} paraquat) and 80% and 65% (for 125 a.i ha^{-1} paraquat) in above-ground biomass over control in resistant populations TF-B1 and TF-B6 respectively. Both populations generated higher numbers of buds (33 to 66% in TF-B1 and 46% to 78% in TFB6) plant^{-1} under two lower doses of paraquat.

Rationale

In Australia, paraquat has been used as a component of "double-knock" tactic to control *Conyza* spp. and other weeds in fallow situations depending on crop rotation, crop choice, weed pressure and other management strategies. It is essential to investigate paraquat resistance in problematic weed species in Australian cropping systems, especially as the determination of herbicide resistance and fitness of resistant weed species is important to develop a proper tactic for the herbicide resistance management (Beckie et al., 2000). A suitable resistance testing bioassay can be used to determine the level of resistance and obtain a glimpse of the potential resistance mechanism (Beckie et al., 2010; Burgos et al., 2013). The estimation of survival and fecundity rates (e.g. seeds) in resistant populations after exposure to herbicides are a true ecological measure of resistance (Vila-Aiub et al., 2015). In herbicide dose response bioassays, some substances, although toxic at higher doses, can be stimulatory at low doses. This biphasic dose–response phenomenon is commonly termed hormesis and it is also characteristic for many herbicides and other phytotoxins (Duke et al., 2006; Cedergreen, 2008).

Objectives

The current study was conducted to primarily confirm the presence of paraquat resistance in *C. sumatrensis* in Australia particularly in cotton farming systems and secondly to determine if any stimulatory response can be observed in the suspected resistant populations when applied at any low doses of paraquat.

Materials and methods

Previously identified three resistant populations of *C. sumatrensis* were selected for this study. Seeds of two resistant (seeds from surviving plants) and one susceptible populations (seeds from the parent plant) were sown on plastic trays (35 cm by 30 cm) pre-filled with potting mix on 15th March 2018. A total of four uniform *C. sumatrensis* seedlings were transplanted from trays to a new pot pre-filled with same type of potting mix at two to four-leaf stages on 10th May 2018. Two weeks later, one uniform seedling was kept for each pot. Plants from three different populations were treated with 8 different paraquat doses (0, 62.5, 125, 250, 375, 500, 625 and 750 g a. i ha^{-1}), where one set of treated plants with four replications was cultivated for 5 weeks in a glasshouse after herbicide application and aboveground biomass was then harvested and dried for two days at 70 °C for the shoot dry weight measurement. A second set of treated pots was kept to observe the sub-inhibitory response of tested biotypes due to low dose application of herbicide, where shoot growth stimulation, biomass and buds plant^{-1} were measured at prescribed days after application of paraquat. Once the above mentioned dose response experiments were terminated, they were repeated in 2018 and 2019 to observe the same resistance response and hormesis phenomenon in *C. sumatrensis* at low doses of photosynthesis (PS I) inhibitory herbicide paraquat.

Paraquat is adsorbed very quickly by plant leaves and blocks photosynthesis by accepting electrons from photosystem I (PSI) (Purba *et al*., 1995). Paraquat was applied on sunny day and leaf chlorophyll concentrations of treated plants were measured. The SPAD meter (SPAD-502 Plus, Konica Miolta, Japan) was used at 15, 40 and 80 DAT, where chlorophyll content was measured at the three different points of three young leaves from top and three old leaves from base of each plant.

Results

Throughout vegetative growth, it was noted that lower-dose treated plants developed more biomass and were always taller than the untreated and the higher-dose treated plants. This can be seen in Figures 1 and 2 by plant height measured at three different days after treatment (DAT). Application of lower doses (62.5 and 125 g a. i. ha^{-1}) stimulated the vegetative growth of resistant populations up to the final evaluation at 80 DAT. Plant height measured at 15 and 40 DAT can be described by the 4 parameters and the same trait measured at 80 DAT can be described by five parameters Brain-Cousens (hormesis; f>0) model respectively. The ANOVA test showed a significantly enhanced plant height at the two lower doses 62.5 and 125 g a.i ha^{-1} when compared to control. The biphasic response was more pronounced for stem elongation in TF-B1 than TF-B6 for two lower doses at 15 and 40 DAT but at 80 DAT stimulation was less in TF-B1 than TF-B6. Hormesis was also more pronounced for both biotypes at 15 DAT than both at 40 and 80 DAT. Data from 15 DAT showed that plant height was stimulated about 43%; 23 % in TF-B1 and %; 14% in TF-B6 over control due to application of two lower doses 62.5 and 125 g a.i ha^{-1}. At 15 DAT, the average maximum response and paraquat concentration was 25 cm (38% over control); 116 a.i. ha^{-1} and 27 cm (29% over control); 96 a.i ha^{-1} for populations TF-B1 and TF-B6 respectively. At 40 DAT, average maximum plant height was stimulated at lower doses of 68 g a. i ha^{-1} for TF-B1 and 46 g a. i ha^{-1} for TF-B6 respectively. At 80 DAT, the average maximum response was 60 and 73 cm and respective herbicide concentrations were 73 and 107 g a. i ha^{-1} for TF-B1 and TF-B6. Initially, TF-B1 showed more stem growth stimulation than TF-B6 but stimulation of TF-B1 did not continue over time and more inhibition occurred at high doses of paraquat than TF-B6.

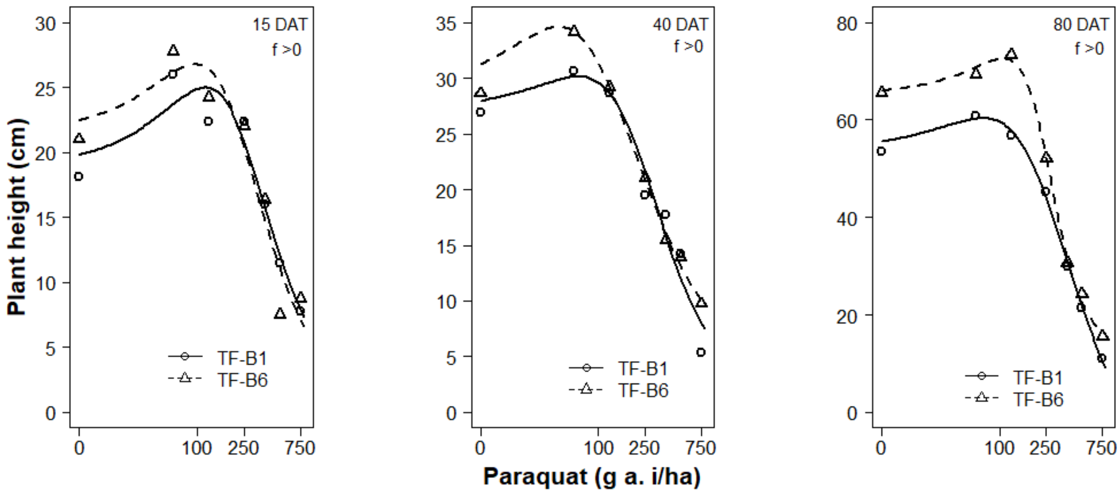


Figure. 1 Dose– response effects of paraquat on plant height of two resistant populations at 15, 40 and 80 days after treatment (DAT). Lines describe the predicted responses (plant height) according to the equations reported in the materials and methods section. Symbols shown are the original means of plant height (cm) of the populations. Mean value was pooled from eighteen observational units. Parameter *f* denotes significant hormesis ($f > 0$) as best fit.

Figure. 2 Responses of a resistant population (TF-B1) of *C. sumatrensis* to different doses of paraquat at 15 days after treatment (DAT).

There was a significant (P<0.001) increase in the aboveground dry biomass at 35 DAT in both resistant populations due to the application of lower doses of paraquat. The hormetic dose range was also located between 62.5 and 125 g a.i. ha^{-1} of paraquat and response was higher in TBF-B6 than TF-B1 (Figure. 3). The hormetic dose range for biomass accumulated was for population TF-B1 with an average y_{max} of 47% (3.1 g) of control (2 g), an average maximum dose (M) of 66 g a.i. ha^{-1} (Table 3). TF-B6 showed more increase in biomass accumulation with an average y_{max} of 85% (3.9 g) of control (2.2 g), an average maximum dose (M) of 71 g a.i. ha^{-1}. The biomass was more stimulated in populations TF-B6 than in TF-B1 with ED_{50} of 383 and 516 g a.i ha^{-1} for TF-B1 and TF-B6 respectively. The hormetically boosted resistant plants of both populations produced, on average, up to 33% (TF-B1) and 46% (TF-B6) more buds at 62.5 g a.i. ha^{-1} paraquat than their untreated plants (Figure. 4). This number further increased to 66% and 78% at 125 at g a.i. ha^{-1} for TF-B1 and TF-B6 respectively. The absolute y_{max} and herbicide concentration values were 131 and 138 pods $plant^{-1}$ for TF-B1 and TF-B6 respectively.

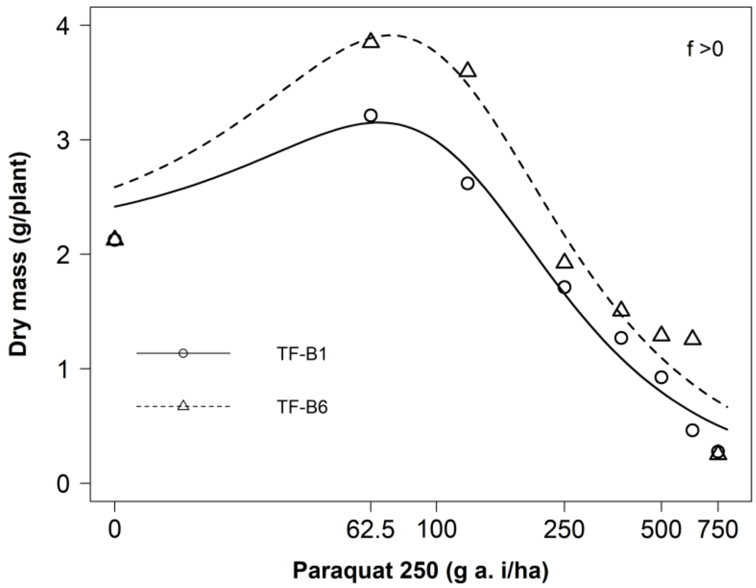


Figure. 3 Mean aboveground biomass and maximum stimulatory response y_{max} in aboveground dry biomass of paraquat treated plants in the two *C. sumatrensis* biotypes at 35 days after treatment (DAT). Mean value was pooled from eighteen observational units. Lines describe the predicted above-ground biomass responses. Symbols shown are the original means of plant biomass.

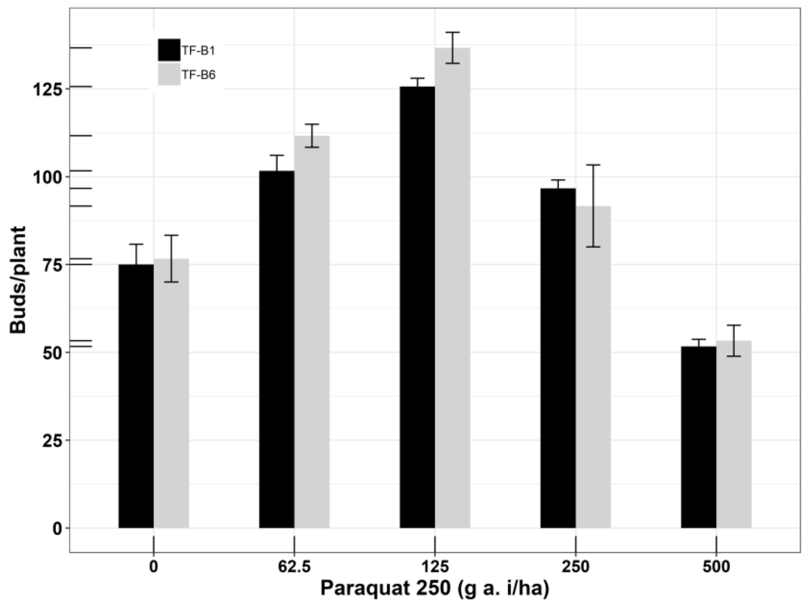


Figure. 4 Mean buds plant^{-1} of paraquat treated plants in two resistant *C. sumatrensis* populations. Error bars presenting mean (± SE). The absolute y_{max} and herbicide concentration values were 131 and 138 pods plant^{-1} for TF-B1 and TF-B6 respectively. The chlorophyll content was significantly (*P*<0.001) different between populations, herbicide doses, new and older leaves. Overall, young leaves from adult plants had

high SPAD values (data not shown). Chlorophyll measured at 80 DAT, showed that application of low concentrations (i.e., 62.5, 125 and 250 g a. i. ha^{-1}) of paraquat had no effect on total chlorophyll content (cm^{-2}) of two resistant populations. A significantly (*P*<0.001) lower number of SPAD values were only recorded after exposed to 500 g a. i ha^{-1} or higher doses of paraquat (Figure. 5). The total chlorophyll content of the susceptible population was drastically reduced (57%) due to application of only 62.5 g a. i ha^{-1} of paraquat. Whereas the application of 500 g a. i. ha^{-1} of paraquat reduced only about 30% of chlorophyll of TF-B1 and TF-B6 which can also be illustrated through visual assessment of the leaf colour (Figure. 6).

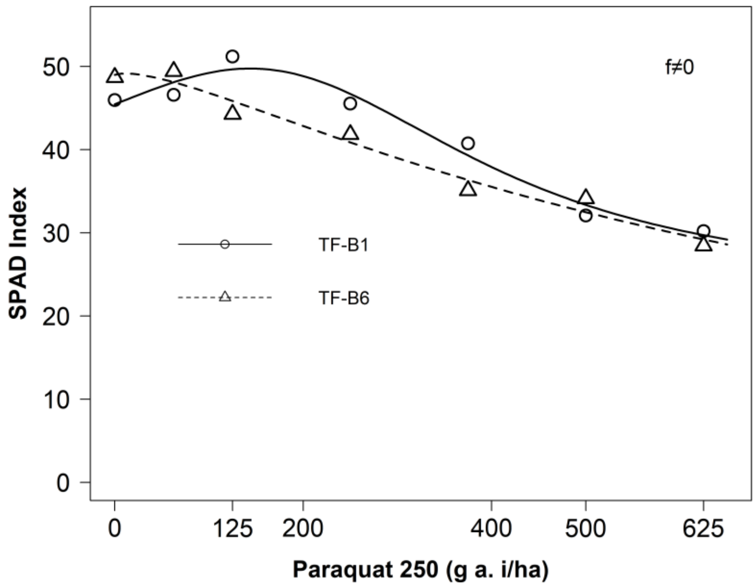


Figure. 5 Dose– response effects on chlorophyll concentration of *C. sumatrensis* after 80 DAT. Lines describe the predicted chlorophyll content or leaf greenness responses. Mean value was pooled from eighteen observational units. Symbols shown are the original means of chlorophyll concentration of the two resistant populations.

Figure. 6 Leaf greenness of TF-B1 after 80 days of paraquat application at different doses (0, 125, 250, 500 and 750 g a. i ha^{-1}) as results of stimulation and inhibition of chlorophyll concentration. Following exposure to 62.5 and 125 g a. i ha^{-1} the abundance of the chlorophyll and leaf greenness did not changes. Chlorophyll concentration was significantly decreased on or after 250 g a. i ha^{-1} paraquat application.

Herbicide Resistance Screening, 2018-2019

Key findings

A total of six ryegrass populations were screened; five populations are resistant to glyphosate and all populations are susceptible to both paraquat and 'double-knock. (glyphosate+paraquat)

A total of 23, 16 and 25 populations of Barnyard grass, Windmill grass and Feathertop Rhodes grass were screened with glyphosate, and 52%, 44% and 48% populations were identified as resistant respectively.

A total of 21 and 14 populations of Feathertop Rhodes and Barnyard grass were treated with four Group A herbicides including three fops (Aryloxyphenoxypropionates) and one dim (Cyclohexanediones). Among group A herbicides, Topik® (clodinafop) was less effective to most of the tested grass populations. A total of seven and five populations of Feathertop Rhodes and Barnyard grass showed resistance to Topik® accordingly.

Grass populations that are developing-resistance or are resistant to Topik® are also resistant or developing resistance to glyphosate except one population of Barnyard grass (S3) and two populations of Feathertop Rhodes grass (D1 and D12).

A total of three populations (out of 11) of Sowthistle are resistant to glyphosate.

Two populations of Bladder ketmia (narrow) are found resistant to glyphosate.

Annual ryegrass

Background

Annual ryegrass (*Lolium rigidum*) is one of the most serious and costly weeds of cropping systems in southern Australia. Many populations of annual ryegrass have developed resistance to both selective and non-selective herbicides. These populations formed following repeated use of glyphosate for winter fallow weed control. This random survey was conducted only in southern cotton system of NSW to determine the extent of glyphosate, paraquat and "double-knock" resistance in annual ryegrass.

Objective: to know the resistance extent and gather baseline data for paraquat and double-knock resistance (glyphosate followed by paraquat).

Materials and methods

A total of six populations of annual ryegrass were collected from cotton fields in southern NSW (Table 1). The fields were randomly surveyed in early 2018. Seeds of six populations were planted in plastic trays and each population had ten plants. The trays were filled with potting mix. Trays were kept in a temperature controlled glasshouse (10 °C minimum, 25 °C maximum) and were watered as required. The ryegrass populations were screened against glyphosate (1.2 L/ha or 0.648 g ai), paraquat-250 (2 L/ha) and 'double-knock' [glyphosate 540 (1.2 L/ha) followed by paraquat (2 L/ha)]. The herbicides were all applied when the plants at growth stage Z12-13 or 3–4 leaf stage (Broster *et al.* 2011; Zadoks *et al.* 1974). All samples were assessed 28 days after treatment by using rating from 0 (no injury) to 100 (died, or injured). Samples were classified resistant if the mean survival percentage was greater than 20%. Developing resistance 10–20% and susceptible <10% survived.

Table 1 Annual ryegrass population collected in Southern cotton system

ID	DOC	Location/Property	GPS coordinates
RB	12.1.2018	Coleambally	NA
S4	6.2.2018	Sandhill edge, fodders field	NA
AR-2	6.2.2018	7 Kms from Whitton, Murrami	34° 27′43.254″S 146° 11′44.9772″ E
CR	6.2.2018	CRN irrigation way	34° 20′31.411″S 146° 12′29.9851″ E
J3	8.3.2018	Conargo	35° 15′48.6504″S 145° 26′35.7828″ E
PW	-	Coleambally	

Results

No populations of ryegrass are resistant or developing resistance to both paraquat and 'Double-knock'. However, out of six populations, one sample was classed as susceptible, two samples were found as developing resistant and remaining three were resistant to glyphosate(Table 2).

Table 2 Glyphosate screened on 6 annual ryegrass populations.

ID	% plant survived after application of		
	Paraquat	**'Double-knock'**	**Glyphosate**
RB	0	0	50 R
S4	0	0	50 R
AR-2	0	0	0 S
CR	0	0	100 R
J3	0	0	80 R
PW	0	0	100 R

(S – susceptible <10% survival; DR –developing resistance 10–20% survival; R – resistance >20%) from random survey 2018–19 season.

Three major grass weed species in cotton systems

Barnyard grass, Windmill grass and Feathertop Rhodes grass

Background

Barnyard grass (*Echinachloa colona*), Windmill grass (*Chloris truncata*) and Feathertop Rhodes grass (*Chloris virgata*) are three of the most problematic grass weeds in Australian cotton system. Populations of these species developed resistance to glyphosate reported by others and in our earlier survey (DAN1402). Group A herbicides are being used extensively for the fallow control of key northern region grass weeds and concern is increasing around the effectiveness of Group A herbicides on these weeds. Several populations of Barnyard grass, Windmill grass and Feathertop Rhodes grass were collected in a random survey in 2017-18 cotton seasons and screened against both glyphosate and group A herbicides.

Objectives: to know the current levels of glyphosate resistance in three major grasses, and are there any difference in the efficacy of different Group A herbicides in controlling principally Barnyard grass, and Feathertop Rhodes grass.

Materials and methods

Glyphosate resistance screening

A total of 23 populations of Barnyard grass, 25 of Feathertop Rhodes grass and 16 populations of Windmill grass were screened against glyphosate under glass house conditions. Seedlings were grown on plastic tray prefilled with potting mix and kept in a glasshouse (temperature 15°C minimum, 30°C maximum). The trays were watered as required. Glyphosate (glyphosate 540 @ 2.4 L/ha) was applied when the plants at growth stage Z12-13 (Zadoks *et al.* 1974). All samples were assessed 28 days after treatment by using rating from 0 (no injury) to 100 (died, or injured). Populations were classified resistant if the mean survival percentage for all replicates was greater than 20%. Developing resistance 10-20% and susceptible <10% survived.

Group A herbicide resistance screening

A total of 4 group A herbicides were tested against different populations of Barnyard and Feathertop Rhodes grass (Table 3). Herbicides were applied at Z13-14 (*Zadoks et al.* 1974) or plant height below bear can height. All samples were assessed 28 days after treatment by using rating from 0 (no injury) to 100 (died, or injured. Populations were classified resistant if the mean survival percentage for all replicates was greater than 20%. Developing resistance 10-20% and susceptible <10% survived.

Table 3. Four different group A herbicides and their respective adjuvant and rates (Cook, 2017; personal communication)

Trt No.	Chemical	Product	Rate (ml or g/ha)	Adjuvant/surfactant
1	**-**	Untreated control		**-**
2	Clodinafop (240 g/L)	Topik®	125mL	Adigor® 0.5L/100L
3	Propaquizafop (100 g/l)	Shogun®	600mL	Hasten® 0.5L/100L
4	Quizalofop-p-ethyl (99.5 g/L)	Targa®	750mL	Non-ionic surfactant (1000gai/L) 0.2L/100L
5	Clethodim (240g/L)	Select®	375mL	Bonza ™1L/100L

Results

Glyphosate resistance

Among three grass species, Feathertop Rhodes grass is more dominant in Darling Downs, and McIntyre (southern Queensland), area. Barnyard grass is more dominant in Macquarie (central), Murrumbidgee, and Lachlan River (southern NSW) areas, whereas Windmill grass pressure was higher in the Macquarie valley region.

The general screening demonstrated that a total of 12 populations of barnyard grass were resistant to glyphosate. Another four populations are developing-resistance (Figure 1 and Table 4). In case of windmill grass, five populations are resistant to glyphosate and two populations are categorised as developing or intermediate resistance. Out of 25 populations of Feathertop Rhodes grass, only one population was resistant. A total of nine populations expressed as developing-resistance.

Group A herbicide resistance

A total of 21 and 14 populations of Feathertop Rhodes and Barnyard grass were treated with 4 different Group A herbicides including three fops (Aryloxyphenoxypropionates) and one dim (Cyclohexanediones). Among the herbicides Topik® (clodinafop) was less effective to most of the populations of both grasses (Table 5). A total of 33% (7 populations) and 35% (5 populations)

populations of Feathertop Rhodes and Barnyard grass showed resistance to Topik® (quizalofop-p-ethyl) respectively. Two populations of Feathertop Rhodes grass also expressed resistance to Select® (clethodim). All tested populations of both species are susceptible to Shogun® (propaquizafop) and Targa® (quizalofop-p-ethyl). Barnyard grass populations which are resistant to Topik® are also resistant or developing resistance to glyphosate except one population (S3).

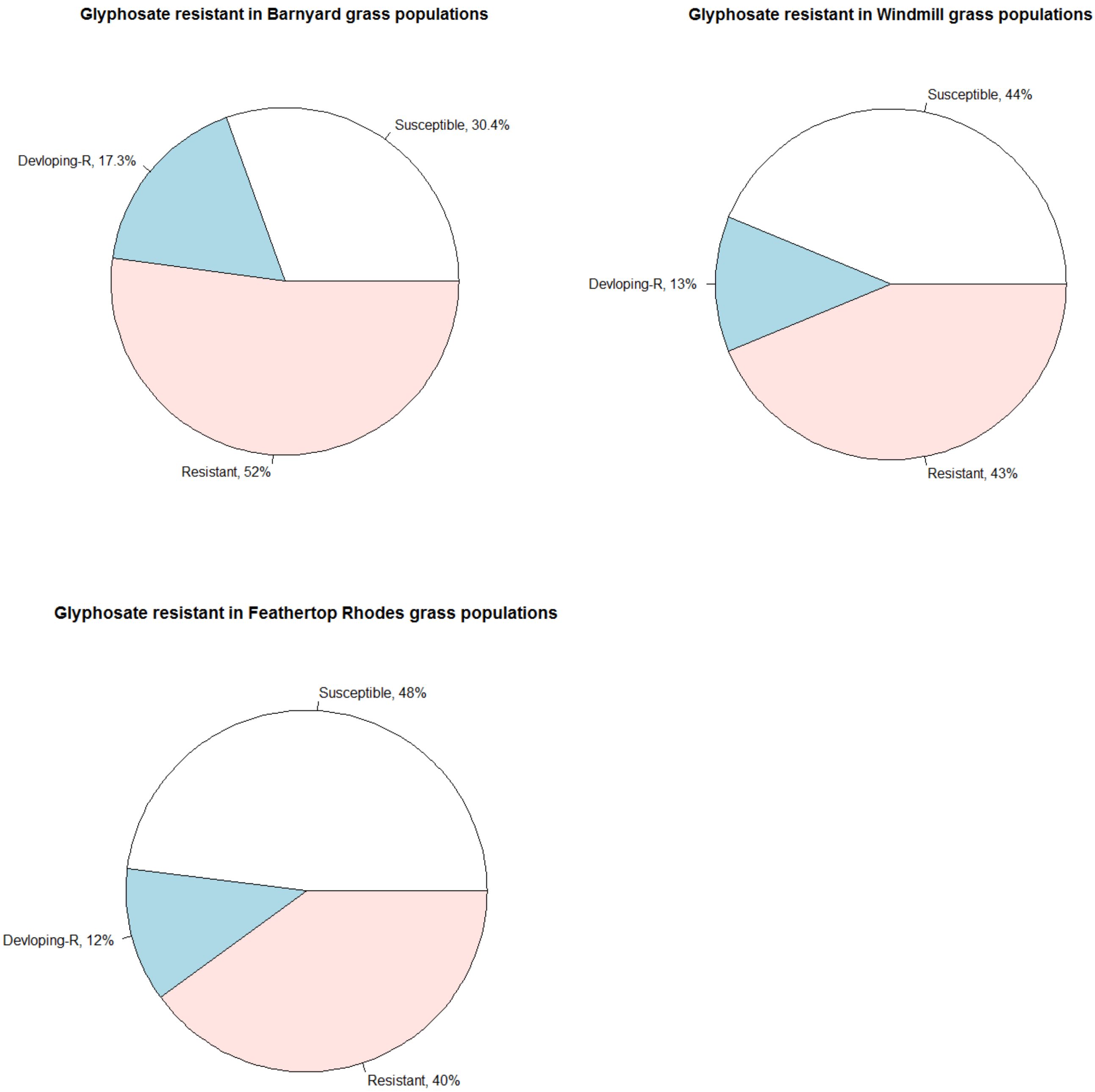


Figure 1 Pictorial summary of glyphosate resistance in populations of three major grasses collected in random survey 2018-19 cotton season.

Table 4 Glyphosate resistance screening in populations of three major grass weed species.

ID	Location/Property	GPS coordinates	Plant survived (%)	Category
Barnyard grass				
BYG5	St George		50	R
BYG7	Goondiwindi		80	R
BYG10	Hay	34° 26' 17.2284'' S 145° 4' 17.7096'' E	30	R
BYG11	Hay	34° 29' 40.2288'' S 144° 44' 53.8224'' E	40	R
BYG7	Boggabilla (QLD)	28° 34' 36.7788'' S 150° 19' 12.72'' E	60	R
BYG3	St George		0	S
BYG-4	St George		30	R
	Boomi	29° 19'.5149'' S 149° 43' 1094'' E	50	R
	Moree	29° 19' 0835'' S 149° 43.2622'' E	30	R
	Moree		50	R
BYG7	St George	27° 26' 52.44'' S 151° 7' 59.124'' E	80	R
J1	Conargo	35° 15' 52.164'' S 145° 29' 37.932'' E	0	S
J3	Conargo	35° 15' 59.796'' S 145° 26' 29.364'' E	20	DR
D5		27° 19' 17.544'' S 151° 7' 57.648'' E	0	S
D6	Cecil Plains Rd		40	R
D8	Millmerran	27° 49' 4.17'' S 151° 16' 23.232'' E	20	DR
D10	Millmerran	27° 46' 55.344'' S 151° 14' 8.7'' E	50	DR

M2	Warren	31° 20' 58.56'' S 147° 30' 4.464'' E	20	DR
M4	Warren	31° 12' 41.148'' S 147° 24' 57.528'' E	0	S
M7	Warren	-	0	S
M8	Trangie		0	S
M9	Warren	32° 8′48.48″S 147° 29′35.592″ E	0	S
M10	Warren		50	R
Windmill grass				
WMG1	Widgelli	34° 20' 33.216'' S 146° 12' 41.6916'' E	0	S
WMG 3	Whitton	34° 31' 39.3096'' S 146° 10' 31.1412'' E	0	S
WMG7	Darlington Point		0	S
D17	Quinalow	2 7° 9' 50.148'' S151° 35' 36.96'' E	0	S
J1			0	S
UT	St George	27° 30' 52.614'' S 148° 22' 24.96'' E	10	DR
M1	Warren	31° 20' 53.808'' S147° 30' 28.26'' E	60	R
M3	Warren	31° 12' 58.356'' S 147° 24' 18.468'' E	10	DR
M5			80	R
M7	Warren	31° 27' 40.104'' S 147° 27' 16.416'' E	60	R
M8	Trangie		70	R
M9	Bogan	32° 8' 48.48'' S 147° 29' 35.592'' E	40	R
M11			90	R
WM9			50	R
D6	Darling Downs		0	S

D7			0	S
Feathertop Rhodes grass				
D1	Evanslea	27° 29' 59.9136'' S 151° 31' 40.8756'' E	10	DR
D4		27° 19' 17.544'' S 151° 7' 57.648'' E	50	R
D6	Darling Downs		0	S
D7	Darling Downs	27° 26' 52.44'' S 151° 7' 59.124'' E	80	R
D11	Cecil Plains	27° 14' 1.896'' S 151° 22' 28.668'' E	0	S
D12	Jondaryan	27° 20' 59.3592'' S 151° 33' 19.9656'' E	0	S
D18		27° 7' 8.004'' S 151° 7' 34.068'' E	0	S
D21	Condamine Plains	27° 45' 11.16'' S 151° 19' 26.4'' E	0	S
M1	Warren	31° 20' 53.808'' S 147° 30' 28.26'' E	10	DR
M7	Warren	31° 27' 40.104'' S 147° 27' 16.416'' E	0	S
M21	Condamine Plains	27° 45' 11.16'' S 151° 19' 26.4'' E	0	S
STG			50	R
	Moree	29° 23' 7195'' S 149.° 59' 6459'' E	50	R
	Moree	29° 34'5267'' S 150°03'4775'' E	10	DR
S3	Whitton	34° 31' 39.3096'' S 146° 10' 31.1412'' E	0	S
FTR3	St George		50	R
FTR4	St George	28° 39' 39.06'' S 148° 25' 38.064'' E	50	R
FTR5	St George	28° 21' 14.1984'' S 149° 40' 28.6284'' E	0	S
FTR6	St George	28° 19' 40.638'' S 150° 16' 52.32'' E	0	S

FTR8	Goondiwindi	28° 41' 49.3548'' S 149° 45' 28.1916'' E	0	S
FTR9	Goondiwindi	28° 22' 22.476'' S 145° 35' 51.288'' E	50	R
FTR13	Dalby	27° 8' 25.44'' S151° 12' 46.26'' E	50	R
FTR14	Dalby	27° 14' 23.28'' S 151° 17' 57.912'' E	40	R
FTR16	Dalby	27° 13' 32.34'' S 151° 9' 41.58'' E	40	R
FTR19	Dalby	27° 8' 52.512'' S 151° 58' 59.16'' E	0	S

(S – Susceptible <10% survival; DR –developing resistance 10–20% survival; R – resistance >20%) from random survey in 2018–19 season.

Table 5. Group A **(**Fops and Dims) resistance screening in populations of Feathertop Rhodes and Barnyard grass.

Feathertop Rhodes						
ID	**Location/ Property**	**GPS coordinates**	**Plant survived (%) after application of**			
			Topik	**Shogun**	**Targa**	**Select**
FTR3	St George		60 R	0	0	0
	Moree	29° 23' 7195'' S 149.° 59' 6459'' E	60 R	0	0	0
FTR9	Goondiwindi	28° 22' 22.476'' S 145° 35' 51.288'' E	40 R	0	0	0
FTR13	Dalby	27° 8' 25.44'' S151° 12' 46.26'' E	80 R	0	0	0
D1	Evanslea	27° 29' 59.9136'' S 151° 31' 40.8756'' E	50 R	0	0	0
D4		27° 19' 17.544'' S 151° 7' 57.648'' E	80 R	0	0	0
D6	Cecil Plains		10 DR	0	0	0
D7	Cecil Plains	27° 26' 52.44'' S 151° 7' 59.124'' E	0	0	0	70

D12	Jondaryan	27° 20' 59.3592'' S 151° 33' 19.9656'' E	30 R	0	0	70
Barnyard grass						
	Moree	29° 19' 0835'' S 149° 43.2622'' E	50 R	0	0	0
	Boomi	29° 19'.5149'' S 149° 43' 1094'' E	60 R	0	0	0
BYG5	St George		40R	0	0	0
BYG7	St George		60 R	0	0	0
S3	Whitton	34° 31' 39.3096'' S 146° 10' 31.1412'' E	60 R	0	0	0

(S – Susceptible <20% survival; DR –developing resistance 20–50% survival; R – resistance >50%) from random survey in 2018–19 season. Only resistant populations are presented in table. **ONLY RESISTANT (Group A) POPULATIONS ARE PRESENTED HERE**

Broad leaf weeds (Sowthistle and Bladder ketmia) resistance screening

Bladder ketmia (*Hibiscus* spp.) is a widespread and troublesome weed found throughout the Australian cotton industry. The species is closely related to cotton plants' phenology and physiology. There are two different species of bladder ketmia, wide leaf (*Hibiscus trionum* var. vesicarius) and narrow leaf (*Hibiscus trionum* var. trionum). Our random survey found that several cotton farms were infested by both species. We assume that glyphosate might have evolved resistance in this species.

Sowthistle is a particular weed of concern as its' biological feature can easily have facilitated its spread across the farming system. The species has become more common over the past 10–15 years.

Objectives: to evaluate the current glyphosate efficacy on populations of these two broad-leaf weed species.

Materials and methods

A total of 11 and eight populations of Sowthistle and Bladder ketmia were treated with test rate of glyphosate. In the glass house, pots were initially filled with commercial potting mix to about 75% of the pot height and covered with a thin layer of fine field soil to improve soil contact with Sowthistle seeds (improved emergence). Seedlings from each pot were transplanted to trays (14 plants/population) and maintained at glasshouse conditions. Seeds of ketmia populations were initially scarified for 20 minutes by H_2SO_4. and placed on trays prefilled with potting mix. The trays of both species were watered regularly to maintain moisture conditions for improved emergence.

When Sowthistle seedlings were at the rosette stage (8–10 cm diameter, 8–10 expanded leaves), they were sprayed with 0.65 kg a.i ha^{-1} of glyphosate, which is commonly used rates for general fallow weed control in Australia (Walker *et al.* 2011). When the ketmia plants are 8–10 cm, they were treated with 0.648 Kg a.i ha^{-1}of glyphosate. The irrigation was turned off for one day and turned back on next day. All samples were assessed 28 days after treatment by using rating from 0 (no injury) to 100 (died, or injured. Populations were classified resistant if the mean survival percentage for all replicates was greater than 20%. Developing resistance 10–20% and susceptible <10% survived.

Results

Eleven populations of Sowthistle were sprayed with glyphosate and three populations were identified as resistant (Table 6).

Out of eight populations of Bladder ketmia, one population of narrow leaf ketmia was identified as resistant and another was as developing-resistant to glyphosate applied before pod development stage (8–10 cm tall) (Table 7 and Figure 1).

Table 6. Glyphosate resistance screening in populations of Sowthistle.

ID	Location	GPS coordinates	Plant survived (%) after application of glyphosate	Status
D23			10	S
M8	Trangie		0	S
J2	Conargo	35° 16' 21.864'' S 145° 27' 57.024'' E	0	S
M6	Warren		10	S
Redhill			0	S
Cowboy	Darling downs		0	S
M9	Warren		30	R
J1	Conargo	35° 15' 52.164'' S145° 29' 37.932'' E	30	R
			40	R
M7	Warren		0	S
D21		27° 45' 19.116'' S151° 13' 56.64'' E	0	S

(S – Susceptible <10% survival; DR –developing resistance 10–20% survival; R – resistance >20%) from random survey in 2018–19 season.

Table 7 Glyphosate resistance screening in populations of Bladder ketmia.

ID	Location	GPS coordinates	Plant survived (%) after application of glyphosate	Status
BK8 (narrow)	Millmerran,	27° 49' 4.17'' S 151° 16' 23.556'' E	0	S
BK10 (narrow)	Pampa	27° 46' 55.3548'' S 151° 23' 34.5804'' E	0	S
M8 (narrow)	Warren	31° 34' 59.376'' S 147° 34' 4.836'' E	0	S
BK21 (broad)	Kurrowah	27° 45' 11.16'' S151° 13' 56.64'' E	0	S
BK21 (narrow)	Kurrowah	27° 45' 11.16'' S151° 13' 56.64'' E	20	DR
BK 22 (broad)	Brookstead,	27° 45' 34.56'' S 151° 28' 5.52'' E	0	S
BK22 (narrow)	Brookstead,	27° 45' 34.56'' S 151° 28' 5.52'' E	10	S
BK8b	Benerembah	34° 23' 36.132'' S 145° 55' 53.9796'' E	40	R

(S – susceptible <10% survival; DR –developing resistance 10–20% survival; R – resistance >20%) from random survey in 2018–19 season.

Figure 1. Ketmia population tested at glasshouse

Conclusion.

Different weed management strategies are needed to be considered to manage glyphosate resistance. Tactics may include: the use of pre- and post-emergence herbicides with different modes of action, greater use of soil-applied residual herbicides, using different mode of action of herbicides in a double-knock, crop interference tactics, other cultural practices and strategic tillage to reduce weed seed banks (e.g. Feathertop Rhodes grass and Sowthistle).

Germination Biology and Ecology

Comparative germination biology study of Dwarf, Green and Redroot amaranth

Key findings

- Dwarf, Green and Redroot amaranth do not show any seed dormancy.
- Temperature has significant influences on seed germination of three tested amaranth weed species.
- All three species can germinate in a wide range of pH solutions however; they prefer to geminate in neutral to alkaline conditions.
- All three species are very sensitive to light and they are photoblastic.
- Dwarf, Green and Redroot are very sensitive to water stress but Green amaranth is more tolerant than the other two species.

The genus *Amaranthus* has many species and of these species, Redroot pigweed, Smooth pigweed, Powell amaranth, Palmer amaranth, Dwarf amaranth, Common waterhemp, and Tall waterhemp are primarily weedy pests in cultivated crops (Horak *et al.* 1994). Our weeds survey in early 2018–19 showed that many cotton farms in Northern Australia are infested by different *Amaranth* species. Understanding the species biology and ecology especially when these species are likely to germinate and emerge aids in planning effective weed management programs. Seed germination is regulated by the interaction of environmental conditions and the state of physiological readiness of seeds.

Objectives: Does each plant species have a specific range of environmental requirements necessary for germination.

Effect of temperature

Experiments were conducted to determine the effects of various constant (15, 20, 25, 30, 35, 40 and 45 °C) and fluctuating day/night temperatures (30/20, 35/25, 40/30 and 45/35 °C) on seed germination of 3 species under 12 hrs light/dark cycle.

Effect of Light

Light effect was evaluated on seed germination under two conditions: a 12 h daily photoperiod (hereafter 'light') and one in continuous darkness (hereafter 'dark') in an incubator for 8 days under fluctuating day/night temperatures of 30/40 °C. For incubation in dark conditions, petri dishes were wrapped in aluminium foil twice, as suggested by Baskin and Baskin (2014). The final germination percentage was calculated for both dark and light conditions after 8 days of seed incubation. The relative light germination (RLG) was measured (Milberg *et al.* 2000) by the following formula:

$$RLG = \frac{Gl}{Gd + Gl}$$

Where *Gl* = germination percentage in light and *Gd* = germination percentage in darkness. *RLG* represents a range of values varying from 0 (germination only in the dark) to 1 (germination only with light).

Effect of pH

The influence of pH on seed germination was determined by using buffer pH solutions of 4 to 10, prepared according to the method described by Chachalis and Reddy (2000). These ranges of pH (ranges 4–10) are those commonly reported in Australian soils (de Caritat *et al.* 2011).

Osmotic Stress

Seeds of three *Amaranthus* species were germinated in the light/dark in aqueous solutions of polyethylene glycol 6000 with osmotic potentials of 0, -0.1, -0.2, -0.4 and -0.8 MPa, prepared by dissolving appropriate amounts of PEG 60002 in deionized water (Michel 1983).

Germination response to soil type and seed burial depth

The effect of burial depth on seedling emergence particularly Dwarf amaranth under two different soil types was evaluated in the glasshouse. Prior to seed burial, 15 cm diameter pots were filled with soil, irrigated with sprinkler system and kept for 14 days in the glasshouse. The germinated seedlings from the weed seedbank were removed prior to seed burial at different depths. A total of fifty seeds of each population were placed on the soil surface of the pots and then covered with soil to depths 0, 2.5, 5, 7.5 and 10 cm. Pots were watered initially with an overhead sprinkler and later with automated sprinkler irrigation system. Seedling emergence was finalised when no further emergence was recorded for a continuous 21 days.

Results

Constant temperature

There was a significant species by temperature interaction for total *Amaranthus* germination (*P*< *0.0001*). All three species failed to germinate a significant number of seeds until the temperature reached 25 °C. The maximum number (83%) of seeds of Dwarf amaranth germinated at constant temperature 35 °C (Figure. 1). Green and Redroot amaranth preferred to germinate at higher temperatures. About 35%of seeds of Green amaranth germinated at 45 °C constant temperature. The high temperature requirement for germination of different *Amaranthus* species was also reported overseas (Baskin and Baskin 1977; Washitani and Takenaka, 1984, Habib and Morton, 1987; Gutterman *et al.* 1992; Guo and Al-Khatib, 2003).

Alternating temperature increased germination of seeds of three species, where Dwarf amaranth and Green amaranth seeds had higher germination under alternating temperatures than under constant temperatures at 25, 35, 30 and 40 °C (Figure. 2). Seed germination of Dwarf amaranth was significantly encouraged by alternating temperature 45/35 °C. A similar trend also observed for Redroot amaranth, indicating altering temperature has significant effect on germination and emergence of these two species.

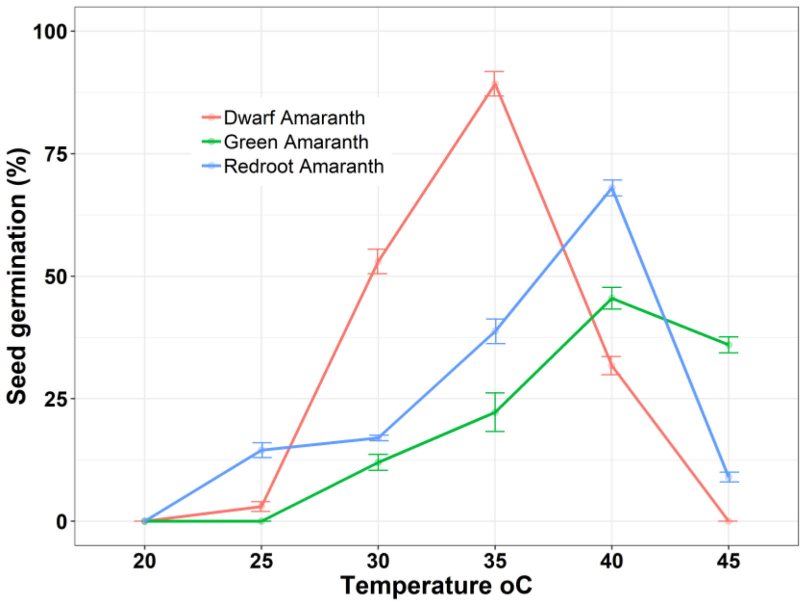


Figure 1. Effect of constant temperatures on seed germination of three *Amaranthus* species collected from cotton farming system in 2017–18 season. Vertical bars represent standard error (± SE).

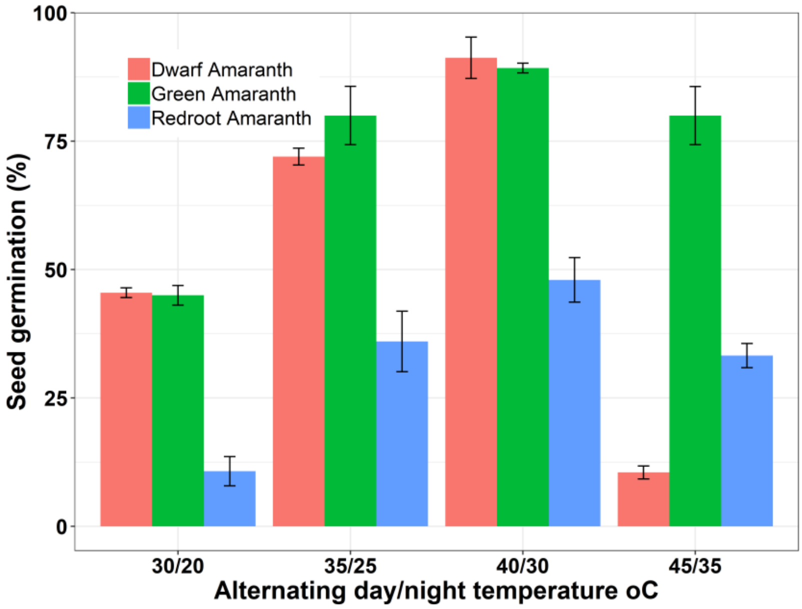


Figure 2. Effect of alternating (day/night) temperatures on seed germination of three *Amaranthus* species collected from cotton farming system in 2017–18 season. Vertical bars represent standard error (± SE).

pH

Seeds of three tested species were able to germinate under a broad range of pH (4 to 10) (Figure. 3). Seeds of all three species prefer to germinate in neutral soils. Nevertheless, a significant proportion of seeds were still able to germinate under alkaline conditions, suggesting that changing pH values by liming or any other cultural practices will not affect their persistence pattern in crop field.

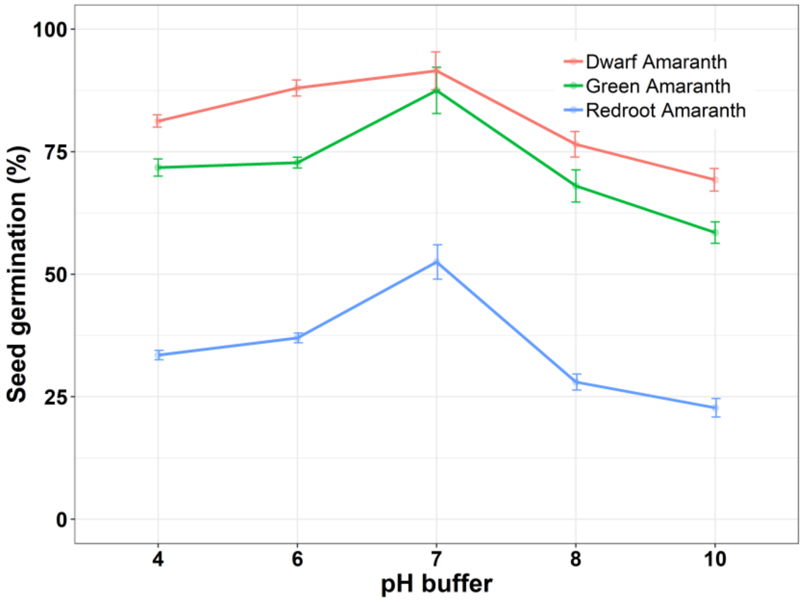


Figure. 3 Effect of pH solutions on seed germination of three *Amaranthus* species collected from cotton farming system in 2017–18 season. Vertical bars represent standard error (± SE).

Osmotic stress

Germination of *Amaranthus* species was significantly influenced by osmotic stress imposed by PEG (Figure. 4). Cumulative seed germination changed at water potential of −0.1 MPa, and after that declined rapidly for both Dwarf and Redroot amaranth. Seed germination of Dwarf and Redroot amaranth were completely inhibited at −0.4 MPa, whereas at −0.8 MPa more than 10% seeds germination were still counted for Green amaranth demonstrating that this species is more water stress tolerant than Dwarf and Redroot amaranth species.

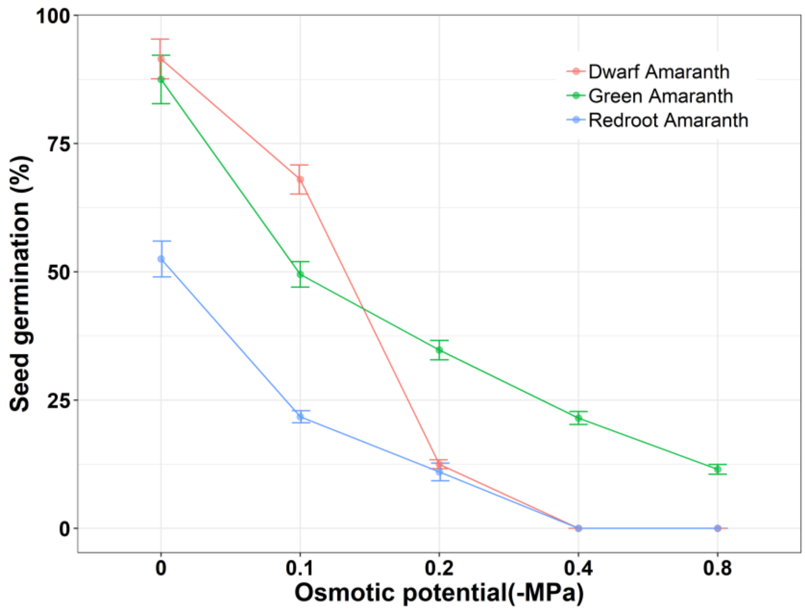


Figure. 4 Effect of osmotic stress on seed germination of three *Amaranthus* species collected from cotton farming system in 2017–18 season. Vertical bars represent standard error (± SE).

Light

There was a significant effect of light ($P < 0.001$) and *Amaranth* species ($P < 0.001$). Seeds of Redroot had at least 48% germination in light conditions and thus were not considered dormant (Table 1). Significantly, a higher proportion of seeds of the three species germinated in the light/dark treatment than in the entirely dark situation. This demonstrates that tested species of *Amaranth* are photoblastic. Species differed significantly ($P < 0.001$) in terms of relative light growth (RLG) and Redroot was more sensitive to light than other two species. The results also indicate that seeds of these species can use light to detect if they are close to the soil surface. This is especially important in the case of small seeded species like *Amaranthus* spp. because small seeds have limited resources and these seedlings would not emerge successfully if germination occurs too deep in the soil (Fenner and Thompson 2005).

Table 1 Effect of light on seed germination of three *Amaranthus* spp of incubated at 40/30 °C alternating day/night temperature for 8 days

Amaranthus species	**Germination trait**		
	Seed germination (%) at light	**Seed germination (%) at dark**	**Relative light germination (RLG)**
Redroot pigweed *(A.retroflexus)*	48[b]	4[c]	0.92[a]
Dwarf amaranth *(A. macrocarpus)*	92[a]	17[b]	0.84[b]
Green amaranth *(A. viridis)*	88[a]	32[a]	0.73[c]

Germination response to soil type and seed burial depth

There was a significant ($p<0.005$) difference between soil types and burial depth to limit the seed germination. The percent emergence of Dwarf amaranth was greater in heavy grey cracking soil than acidic red soils ranging 0–47% and 0–36% respectively (Figure. 5). However, there was no significant ($p>0.05$) difference between populations for seed emergence between both soil types and burial depths. The maximum mean number of seed emergence occurred at 0.5 cm depth in both soils with 47% and 36% emergence for grey and acidic red soil respectively. The reduced germination at higher burial depth might be due to poor gas exchange, poor light, and lower temperature, conditions not suitable for smaller seeds of Dwarf amaranth.

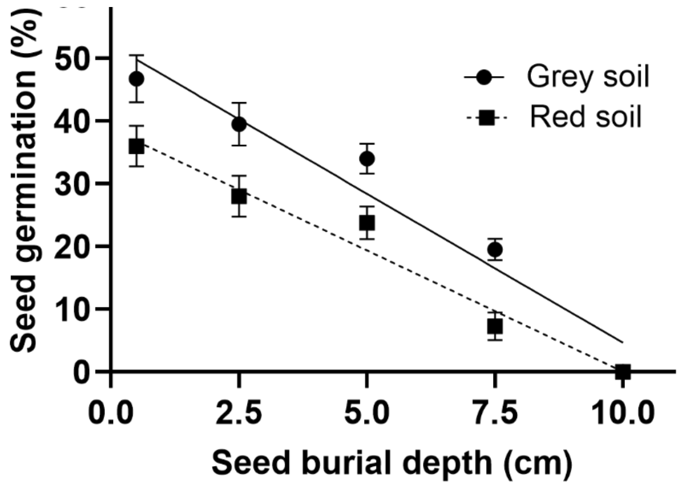


Figure. 5 Effect of burial depth on seed germination of three Dwarf amaranth collected from cotton farming system in 2017–18 season. Vertical bars represent standard error (± SE).

Weed Ecology

Fitness outcomes in glyphosate-R and –S population in Barnyard grass.

Rationale

Glyphosate is the principal form of weed control in Australian cotton systems and therefore this system often exerts strong selection pressure for herbicide resistance. Herbicide resistance is an inherited ability of a plant to survive an application of herbicide at its labelled use rate. A dose–response experiment is often conducted to determine the level of resistance and obtain a glimpse of the potential resistance mechanism. Resistance beyond the recommended dose is no longer important to the grower, but it is relevant to researchers because the resistance level provides clues to resistance mechanism(s), the understanding of which helps in designing management strategies (Burgos et al. 2013). Survival rates in herbicide-exposed populations do provide important information but do not give information about the evolutionary dynamics of a resistance-endowing trait in a weed population under herbicide selection (Vila-Aiub MM et al. 2015).

Objectives

This study identified two phenotypic (susceptible S versus resistant R) lines from within two segregating glyphosate-resistant populations of Barnyard grass *E. colona* and evaluated their fitness through estimation of survival, growth and reproductive rates to characterise the level of resistance, for glyphosate.

Materials and methods

Identification and selection of glyphosate-susceptible and -resistant individuals from within population

In order to identify the susceptible (S) and resistant (R) plants, two resistant populations (2B21 and 2B37) were selected from our earlier resistance screening conducted in 2016–17. A plant cloning technique was applied in two populations (Vila-Aiub et al., 2003; 2011; 2015), where the phenotypic identification after glyphosate selection of S plants within a segregating R *E. colona* population. This approach was conducted outdoors in the enclosed outdoor shade house at Wagga Wagga Agricultural Institute (WWAI) during the normal growing summer season

(2017/2018). For selection of R plants, acid scarified seeds were germinated in plastic tray pre-filled with potting mix. Seedlings at the 2–3-leaf stage were treated with 2160 g ha^{-1} of glyphosate (Roundup PowerMax®; Nufarm, 540 g L^{-1}) (Vila-Aiub et al.2015). Plants were maintained outdoors after treatment and irrigated as required. Plant survival was recorded three weeks after glyphosate treatment, and surviving, growing plants were classified as R plants. Those plants that appeared to be alive without displaying vigorous new growth were unclassified and discarded. For selection of S plants, plants were cloned and numbered. At the 3–4-tiller stage, seedlings were removed from the plastic trays and two tillers per plant (one clone) were excised. These clones were trimmed to 1 cm of shoot material, re-potted and numbered accordingly. When the clones reached the 2–3-leaf stage, they were sprayed with 300 g ha^{-1} of glyphosate. In all cases, glyphosate was applied using a laboratory spray cabinet with a using an automated laboratory sized cabinet sprayer with a moving boom from a flat fan nozzle at 300 kPa pressure.

Identified S (selected from the untreated corresponding cloned plants) and R (selected from the treated surviving individuals) plants were individually transferred into bigger pots (24.5 cm in diameter and 27.5 in height) containing a potting mixture. Plants were irrigated as necessary. A significant distance was maintained between R and S individuals to prevent pollen contamination from other sources. Seeds from individual plants were harvested, cleaned and stored in separate paper bags for further verification.

Characterisation of populations to GR and GS by glyphosate discriminatory dose assay

Seeds of R individuals of both populations were sown in plastic pots containing potting mix. A thinning operation was done at 2–3 leaf stage to minimise the effect of different plant densities on reproductive traits. A total of 50 plants were kept in the trays and the remaining plants removed. Seedlings from the R and S phenotypes were treated with glyphosate at doses of 0, 62.5, 135, 270, 540, 1080, 2160 and 4320 g ha^{-1}. Pots were arranged in a completely randomised design. Glyphosate effects on plant survival, above-ground vegetative growth and seed production were determined. Survival and vegetative biomass were assessed four weeks after treatment, the seed mass and number were quantified at the end of the growth period (12 weeks after transplanting). Quantitative differences in glyphosate resistance levels in terms of either survival, vegetative or reproductive traits between the S and R phenotypes were calculated as a resistance index (RI) = LD_{50R}/ LD_{50s};

where LD denotes the Lethal Doses which killed/reduced 50% plants/mass for the S and R phenotypes.

Results

Selection of glyphosate-susceptible and glyphosate-resistant *E. colona* phenotypes

When exposed to the glyphosate dose 540 g a.i ha^{-1}, more than 80% and 70% plants of R phenotypic lines of 2B-21 and 2B-37 respectively survived (Table 1).

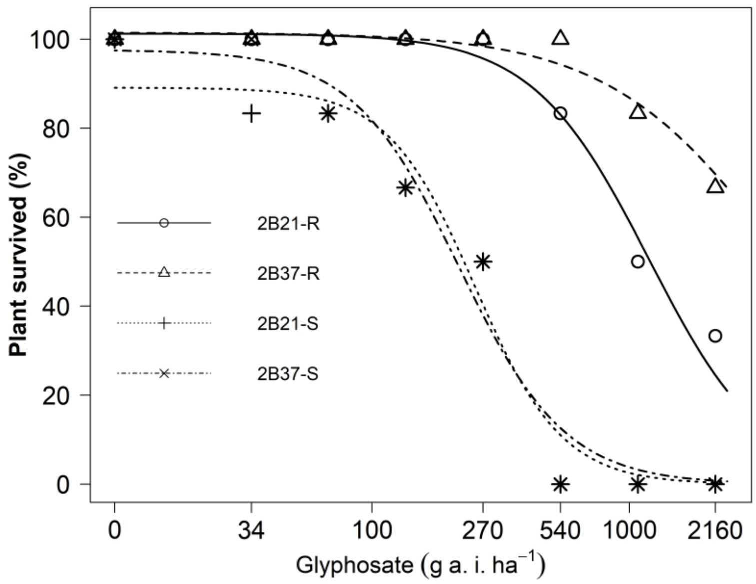


Figure. 1 Dose–response assay of glyphosate resistant and susceptible plants of two populations of Barnyard grass. Lines describe the predicted survival and symbols shown are the original means of plant survived (% of control) of the four phenotypes.

Dose response study revealed that plant survival, vegetative biomass, phenological traits and fecundity differed significantly (p<0.005) between selected S and R phenotype within population and also between populations.

Table 1 Percentage of plants that survived under typical Glyphosate 540 g a. i ha^{-1} dose

Phenotype	Plant survived (%)
2B21-R	80
2B37-R	70

Plant mortality

The herbicide rates calculated to cause a 50% (ED_{50}) plant mortality for the four phenotypes of two populations of *E. colona* are shown in Table 2 and the dose response curves for surviving plants are shown in Figure 1. The susceptible phenotypes of 2B21-S and 2B37-S were completely killed by 540g a. i. ha^{-1} of glyphosate. The ED_{50} value for 2B21-S and 2B37-S were 250 and 217 g a. i. ha^{-1} respectively, those are significantly ($P<0.001$) lower than the ED_{50} values of 1190 and 3918 g a. i ha^{-1} calculated for corresponding resistant phenotype within populations 2B21-R and 2B37-R respectively. Hence, the 540 or more rate of glyphosate had little effect on these two R phenotypes of both populations. By comparing the ED_{50} values of the four phenotypes from tested populations, it was estimated that R phenotypes 2B21-R and 2B37-R are about 5 and 15 times more resistant to glyphosate than their corresponding S phenotypes 2B21-S and 2B37-S respectively. The resistant phenotype 2B37-R was more resistant than 2B21-R.

Table 2 Estimated ED_{50} from dose response survival study

Phenotype	**ED_{50} (g a.i ha^{-1})**
2B21-R	1190
2B21-S	250
2B37-R	3900
2B37-S	217

Above-ground biomass

There was a stimulation of biomass production 12 weeks after glyphosate application in both S phenotypes due to the application of lower doses of glyphosate and the stimulation dose range was located between 34 and 67.5g a.i ha^{-1} of glyphosate (Figure. 2). The stimulation dose range for biomass accumulated was broader in both S phenotypes than R phenotypes and about 10% stimulation occurred in both S phenotypes at 34 g a.i. glyphosate dose. The stimulation dose range however ceased and biomass production was drastically reduced at glyphosate dose at 100 g a.i. ha^{-1}. Stimulation slightly occurred in 2B21-R or no stimulation observed in 2B37-R but both R phenotypes of these did kept overall more biomass production. This suggests that R phenotypes do not show any over stimulation for biomass production at lower doses than S phenotypes but R phenotypes can maintain more biomass throughout their lifecycle.

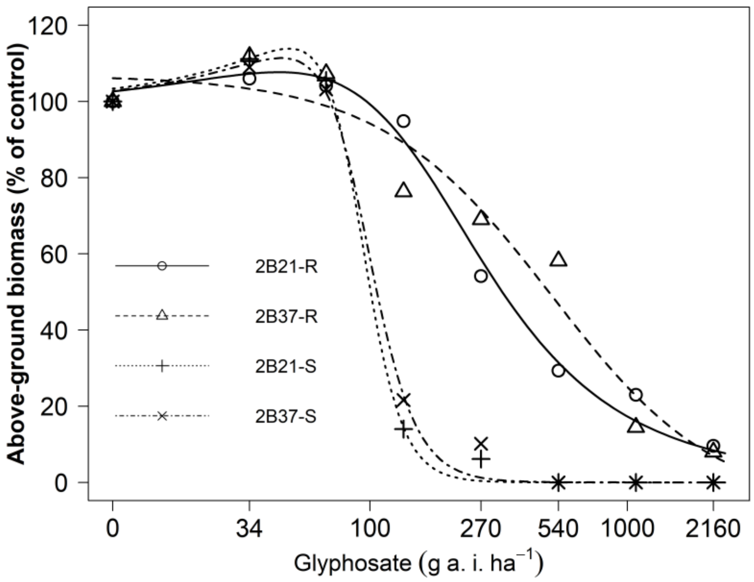


Figure.2 Above-ground biomass glyphosate-resistant and susceptible phenotype of two barnyard grass populations. Lines describe the predicted survival responses and dots are observed mean value from experiment.

Spike initiation and distance between spikes

There was significant effect between herbicide doses (0, 270 and 540 g a.i. ha^{-1} glyphosate) to regulate the spike initiation in R phenotypes. In the absence of glyphosate, plants from R phenotypes of 2B21 population required 43 days (from days of herbicide application) for first spike initiation but in the presence of herbicide (540 g a.i g ha^{-1}) it took 50 days (Figure. 3). While plants of R phenotypes of 2B37 population took 41 and 57 days for absence and presence of herbicide respectively indicates there is cost for spike initiation in R phenotypes.

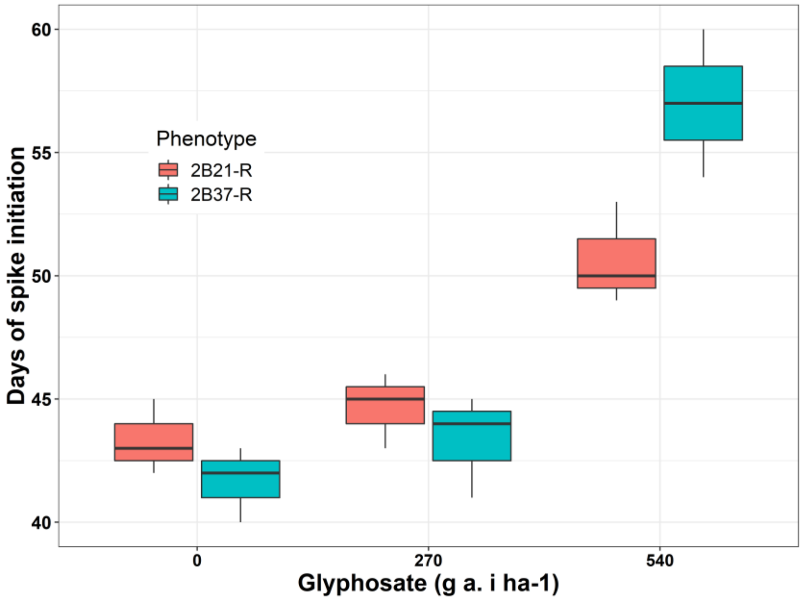


Figure. 3 Days for spike initiation in R phenotypes in the absence and presence (540 g a.i ha^{-1})

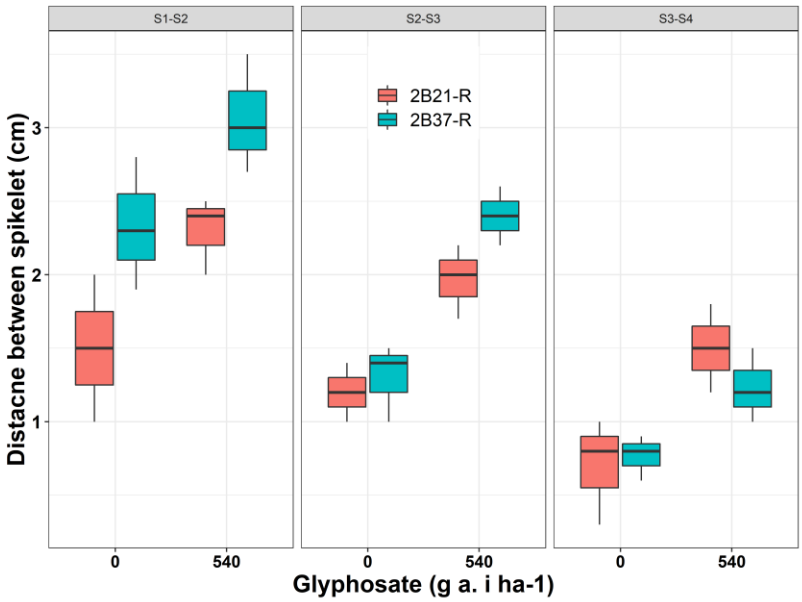


Figure. 4 Distance between spikelets within spike in R phenotypes in the absence and presence (540 g a.i ha^{-1})

The measured distance between first appeared spikelet and second spikelet (S1-S2), second and third (S2-S3) and third and fourth (S3-S4) was significantly (p<0.05) higher in glyphosate treated (540 g a. i ha^{-1}) than non-treated (0 g a. i ha^{-1}) plants from R phenotypes of both populations. For instance S1-S2 values for 2B21-R is 1.2 and 2.3 cm in the absence and presence of glyphosate and similarly 2.3 cm and 3.1 cm for 2B37-R (Figure. 4).

Spikes/plant and seeds/plant

The plants from R lines of 2B21 and 2B37 populations generated 30% and 37% fewer spikes than susceptible plants in the absence of herbicide (Figure 5). The spike numbers of S phenotypes were higher than R phenotypes in the absence or low doses of herbicide (0 to 67.5 g a.i ha^{-1}) but declined rapidly with increased herbicide rates and there was no spike formation in both S phenotypes after exposure to herbicide dose 540 g a.i. ha^{-1}. Both R phenotypes produced more spikes than S phenotypes in the presence of herbicide or increased doses (135 g a.i. ha^{-1}).

Interestingly, seed production declined with increasing herbicide rates in genetically dissimilar phenotypes of both populations. Overall R lines produced double the seeds at each dose of glyphosate than S phenotypes (Figure. 6). These differences were significant, indicating that there is no cost of glyphosate resistance in R phenotype for spike production in the presence of glyphosate.

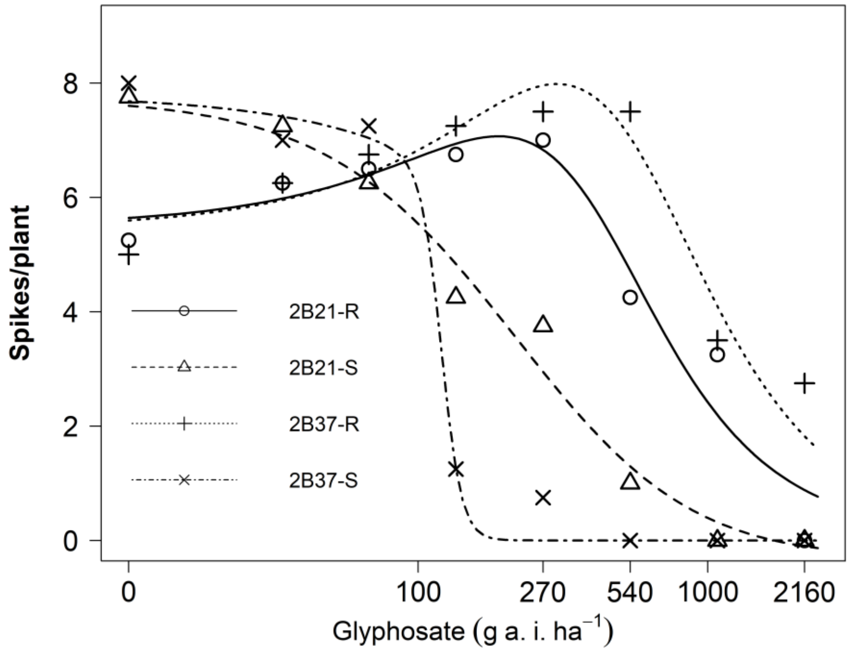


Figure.5 Spike/plant under dose–response assay of glyphosate-resistant and susceptible phenotype of two Barnyard grass populations. Lines describe the predicted survival responses and dots are observed mean value from experiment.

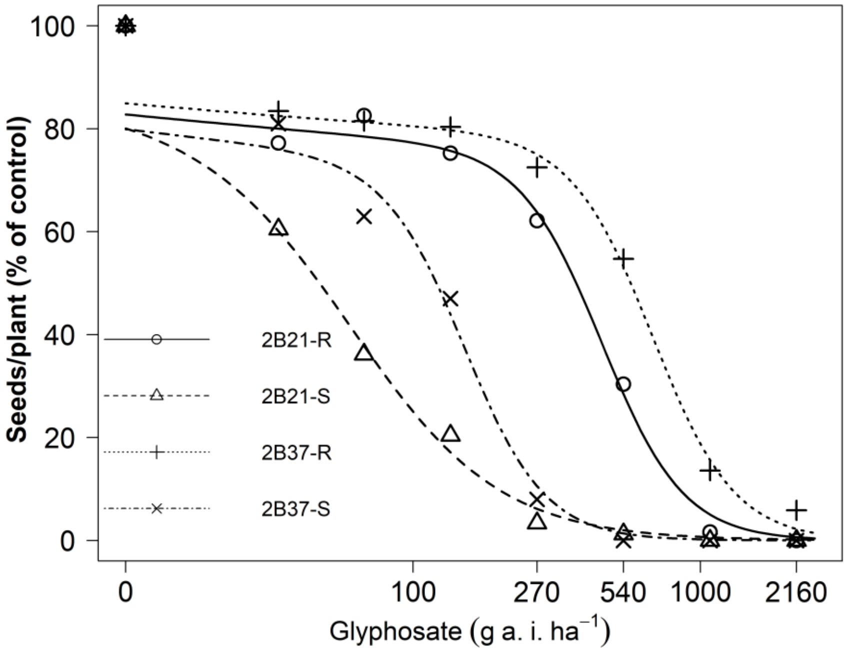


Figure.6 Seeds/plant under dose–response assay of glyphosate-resistant and susceptible phenotype of two Barnyard grass populations. Lines describe the predicted survival responses and dots are observed mean value from experiment.

Table 3 Survival rate, fecundity and fitness of R and S phenotype of two barnyard grass populations

Herbicide dose (g ai /ha)	Phenotype	Survival rate	Fecundity	Fitness
270	2B21-R	1	0.62	0.62
	2B21-S	0.5	0.03	0.015
	2B37-R	1	0.74	0.74
	2B37-S	0.5	0.08	0.04
540	2B21-R	0.83	0.3	0.249
	2B21-S	0	0	0
	2B37-R	1	0.55	0.55
	2B37-S	0	0	0

Fecundity

At the recommended glyphosate dose of 540 g ha^{-1}, the estimated survival rates in the 2B21-S, 2B21-R, 2B37-S and 2B37-R R phenotypes were 0, 83, 0 and 100% respectively (Table 3). Compared with plants not treated with glyphosate, all (100%) of S plant seed production was reduced at the recommended or 540 g a.i ha^{-1} glyphosate dose, whereas for the R phenotype the reduction in individual seed production was 70% and 45% for populations 2B21 and 2B37 respectively (Table 3).

These findings made it possible to estimate the fitness for both the R (W = 0.25) and R (W =0.55) phenotypes relative to the fitness under no herbicide treatment (W =1) (Table 3). These results showed that 30% and 55% number of seeds would be returned to the soil seed bank. At a lower glyphosate dose (270 g ha^{-1}), more S and R individuals survived (50, 100, 50 and 100% respectively) (Table 3) than for those plants treated with glyphosate at 540 g ha^{-1}. Seed number production of the S and R phenotypes was reduced by 38 and 26% for R phenotypes of 2B21 and 2B37 respectively. When the glyphosate dose was doubled (from 270 to 540 g ha^{-1}) the fitness of the susceptible plants was nil and decreased approximately one and half fold (from 0.74 to 0.55) and two and half (0.74 to 0.25) for R phenotypes of 2B21 and 2B37 respectively.

Conclusion

According to evolutionary and ecological theory, our findings agree that herbicide-resistant plants of barnyard grass will be less fit than wild type in the absence of herbicide (glyphosate) and in the absence of glyphosate, backward selection can occur. This means that without glyphosate applications, the susceptible plants of barnyard grass, as the fittest population, may eventually dominate in the field. While resistant plants of same population may dominate in presence of herbicide but depends on glyphosate doses.

Pre-emergent/Residual Weed IWM demonstration 2018/19 Whitton, NSW

Eric Koetz, Md Asaduzzaman, and Graham Charles

Key findings

At Irrigation Research Extension Committee, Whitton

Weed numbers were lowest when a mixture of different modes of action was applied. The addition of glyphosate to: Diuron®, Bouncer®, or Rifle® reduced weed numbers compared to Glyphosate applied alone.

A number of cotton traits including open bolls/plant, yield (bales/ha), lint/plant, lint weight, seed cotton/ha, are higher in the Glyphosate with Bouncer® combination than other treatment especially solely glyphosate based weed control approach.

Objectives

The aim is to highlight to growers that a diverse approach to weed control with reduced reliance on glyphosate will help protect and prolong its usefulness. A side issue raised by growers is around crop establishment and plant stand issues when using pre-emergent and residual herbicides especially in the southern valleys.

Materials and methods

The trial used a mimic grass weed (Japanese millet) to reflect herbicide resistant grass weeds. Half the plots were sown with the mimic weed prior to the application of herbicide treatments.

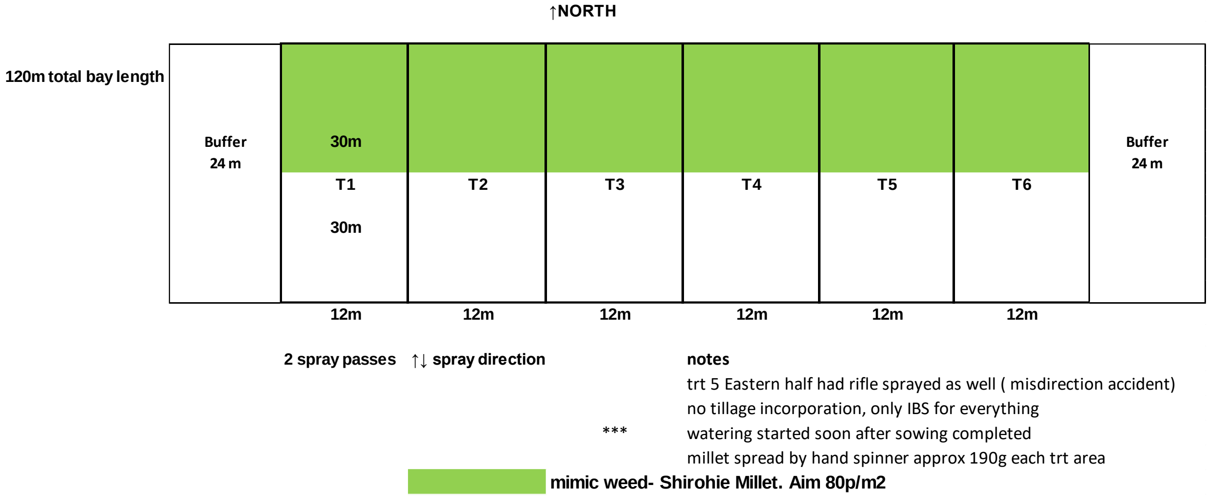
Irrigated system (IREC, Whitton)

Treatments

1. Glyphosate (Weedmaster DST®) (1.5L/ha) only system; pre plant and post emergent Over The Top (OTT) as required, **+/-** mimic weeds to show "resistant" scenario
2. Glyphosate (1.5L/ha) pre plus Glyphosate (1.5L/ha) + Bouncer® (1L/ha) OTT post @ 4 nodes
3. Glyphosate (1.5L/ha) pre plus directed Glyphosate (1.5L/ha) + Rifle® (2L/ha) @ 4 nodes

4. Rifle® (1.9L/ha) pre plant Post Sowing Incorporation (PSI) plus Glyphosate (1.5L/ha) + Bouncer® (1L/ha) OTT @ 4 nodes
5. Diuron® (1.5kg/ha) pre-plant (PSI) and Glyphosate (1.5L/ha)

6. Conventional; Rifle® (1.9L/ha) (PSI) + Glyphosate (1.5L/ha) + Gesagard® 2.5L/ha (applied as layby)



Treatment placement and spray condition: All herbicide treatments were applied after sowing the mimic weed seeds (millet) sown to target 80 plants/m^2) on 12th October 2018. The herbicides were sprayed with a 6 m long boom. The average wind speed was 17.2 km/hr. The humidity and temperature was 34% and 22-24 °C respectively. The post-applied treatments were applied at 4 nodes stage.

Cultivar and seeds sowing: Bollgard3 (cv.748 B3F) and seeds were sown after soil applied herbicide treatments. Sowing rate was 16 seeds/m.

Handpicking: One metre of a single row of cotton was handpicked from the middle of each plot on 16th April 2019 to assess yield attributes and yield in response to the different herbicides treatments. One meter long area was randomly selected for each herbicide treatment and number plants, total bolls (green and matured) were counted. The collected bolls were air dried to @ 10% moisture and hand ginned at Yanco Research Station.

Results

Weediness: Initial weediness score (prior to post treatment placement) demonstrated that cotton seedlings had no injury and the most weed infested plot was Glyphosate alone (Figure. 1). Pre-emergent herbicides did a good job of reducing initial weed numbers prior to the first in-crop glyphosate spray.

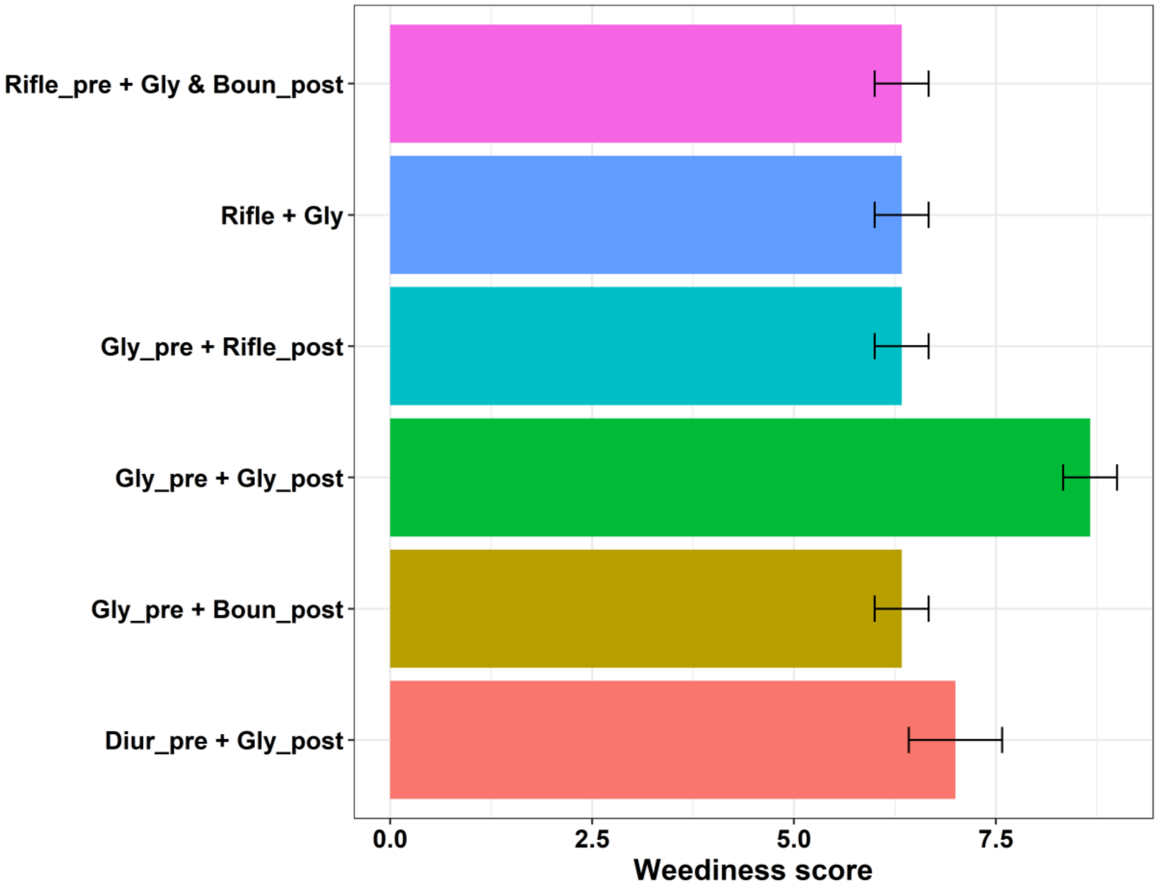


Figure 1. Initial weediness score (1–10) at IREC site. Bar represent mean weediness score (±SE)

Number of plants/m and bolls/plant: One sample "*t* test" revealed that there were significant differences between herbicide treatments in response to both number of plants/m and total numbers of bolls/plant (Figure 2). The highest numbers of plants per unit area was recorded under the treatment of *Glyphosate (1.5 L/ha) as pre plus directed Glyphosate (1.5L/ha) + Rifle® (2 L/ha) as post*, however the maximum numbers of bolls/plant was counted in commercial practiced followed by *Glyphosate (1.5 L/ha) as pre plus directed Glyphosate (1.5 L/ha) and Bouncer®* as *post* treatment. The other treatment such as *Rifle® (1.9 L/ha) pre plant plus Glyphosate (1.5 L/ha) + Bouncer®* 1 L/ha) *as post* also produced highest numbers of Bolls/plant. The number of plants might be varied due to other factors including seed viability, sowing depth and so on but it can be suggested that Bouncer® had no effect on bolls formation at later growth stage of cotton.

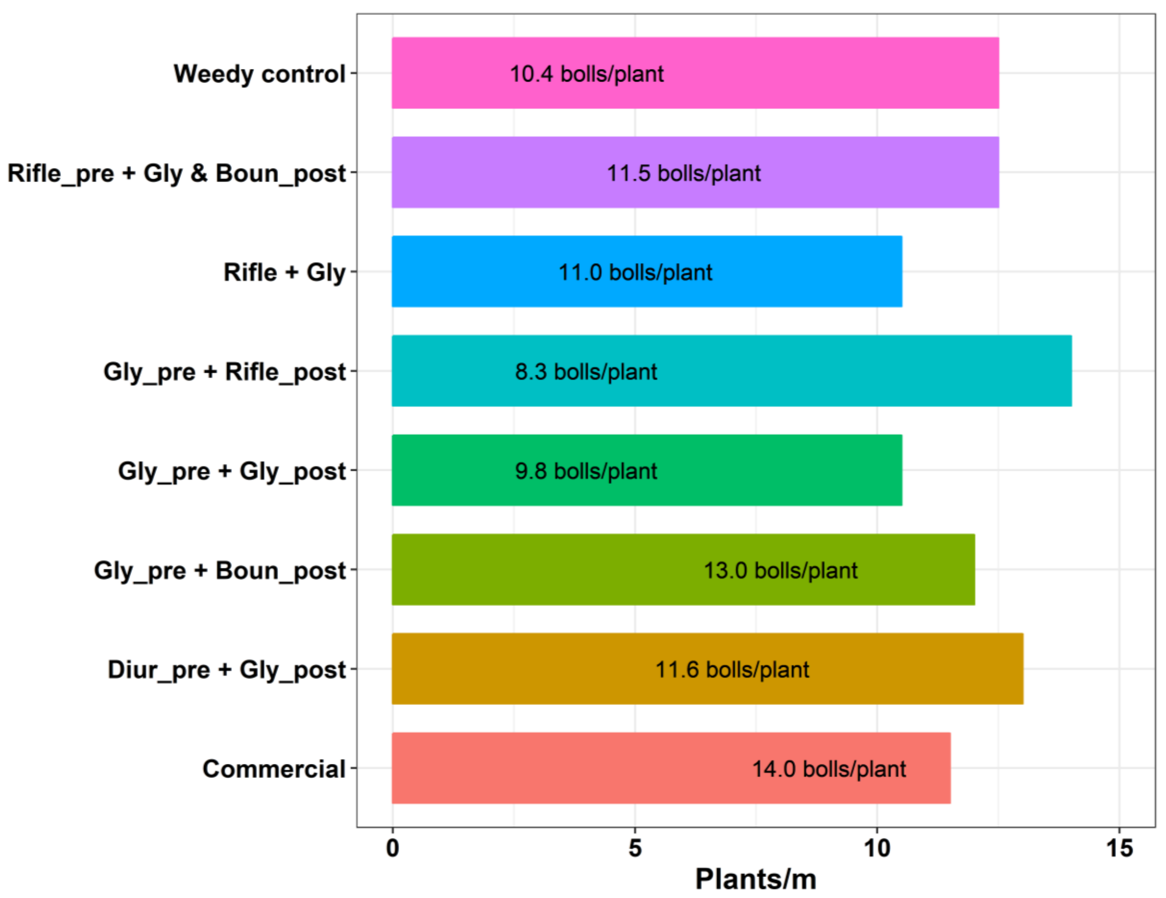


Figure 2. The number plants/m and number bolls/plant (value inside the bar)) at IREC site.

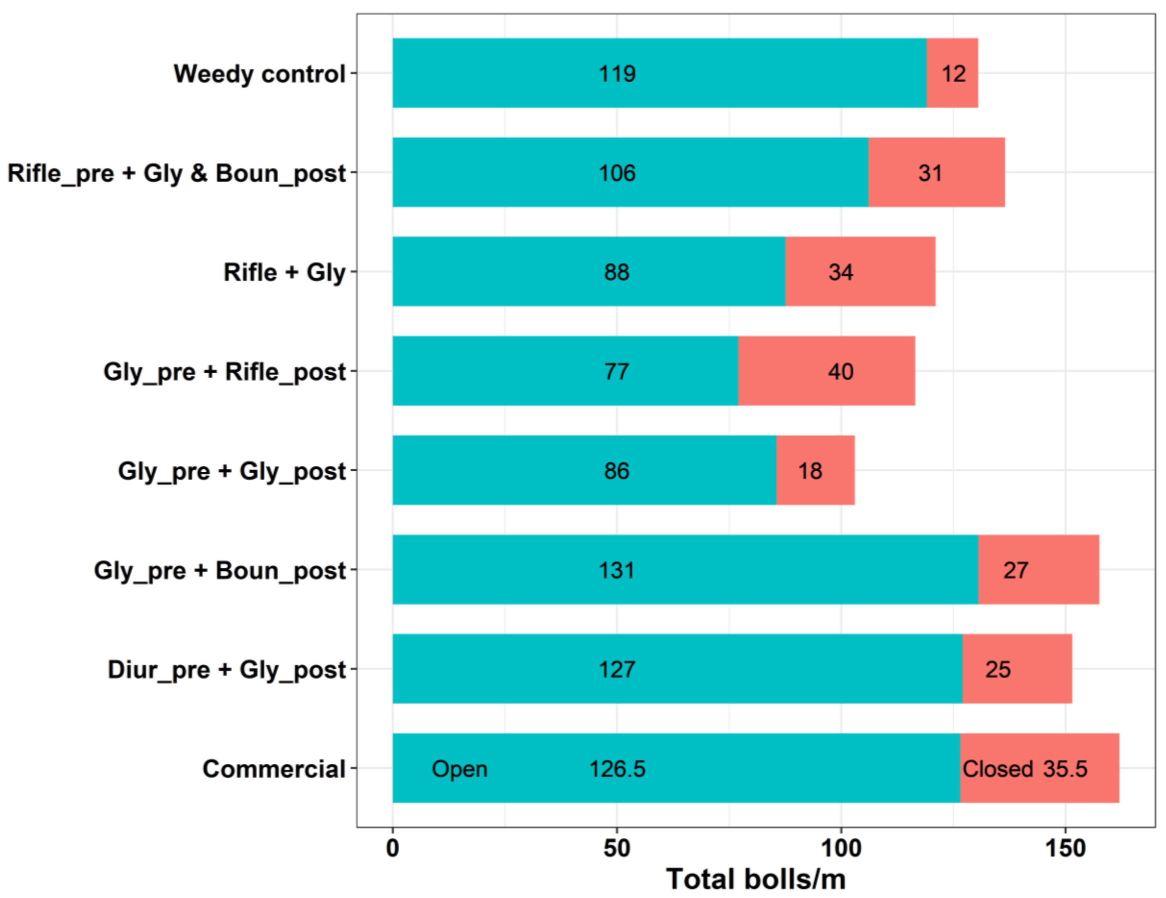


Figure 3. The total bolls (Open + Closed)/m at IREC site.

The number of Open and Closed bolls: The highest number of total bolls/m was found at *Commercial* practice followed by *Glyphosate (1.5 L/ha) as pre plus directed Glyphosate (1.5 L/ha) and Bouncer®* as post treatment (Figure 3). It was interesting that the maximum numbers of opened bolls (opened bolls 131 + closed bolls 27 = Total bolls 158) was recorded under *Glyphosate (1.5 L/ha) as pre plus directed Glyphosate (1.5 L/ha) and Bouncer®*. The maximum numbers of closed bolls was found at treatment *Glyphosate as pre and Rifle® as post herbicide*.

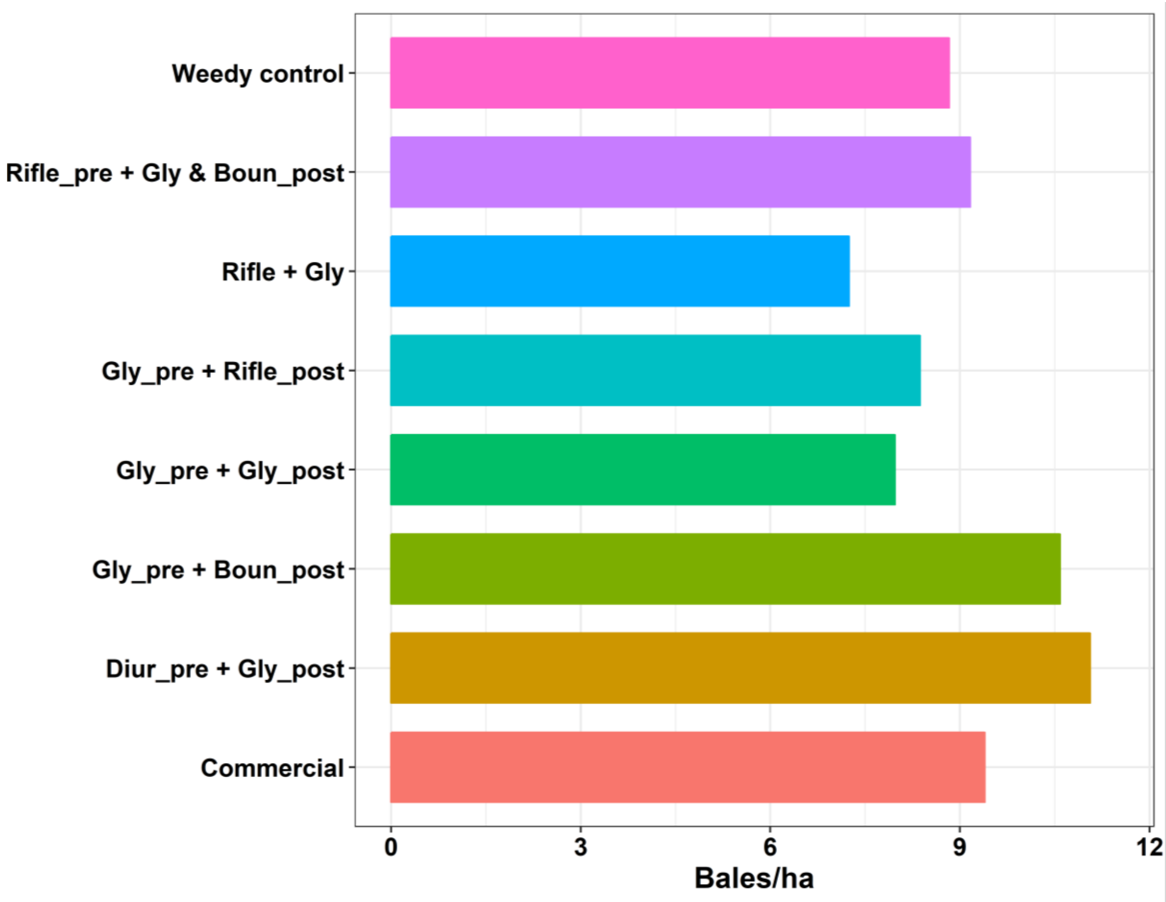


Figure 4. Yield (bales/ha) under different herbicide treatments at IREC site.

Table 1 Different yield attributes of cotton under different herbicide treatments

Treatment	Lint weight (g)	Seed Cotton (kg/ha)	Lint/plant (g)	Lint/boll (g)	Bales/ha (+green bolls)
Gly_pre + Gly_post	174.29	4218.85	17.23	2.121	9.60
Gly_pre + Bouncer®_post	174.95	5533.95	20.02	1.84	12.77
Gly_pre + Rifle®_post	170.81	4478.75	13.57	2.47	12.66
Rifle®_pre + Gly & Bouncer®_post	169.37	4930.75	16.64	1.96	11.79
Diuron®_pre + Gly_post	172.68	5828.50	19.31	1.98	13.19
Rifle® + Gly	171.85	3827.80	15.66	1.88	10.02
Weedy control	172.30	4776.70	16.03	1.68	9.68

Yield (bales/ha): The handpicking harvest data showed that *Diuron® (1.5 kg/ha) as pre and Glyphosate (1.5 L/ha)* as post (Figure.4). While other herbicide treatments produced less than 10 bales/ha.

The other yield attributes including lint/plant, lint weight, seed cotton/ha, total bales with closed bolls are higher at Glyphosate with Bouncer® than other treatments especially compared to solely glyphosate based weed control approach (Table 1).

Correlation among traits: The correlation matrix (Figure 5) shows that herbicide treatments are positively correlated with bolls/plant (e.g. Bouncer® treatment has more bolls/plant). The number of plants positively correlate with actual lint, and bales/ha. The seed cotton/ha significantly correlated with bolls/plant.

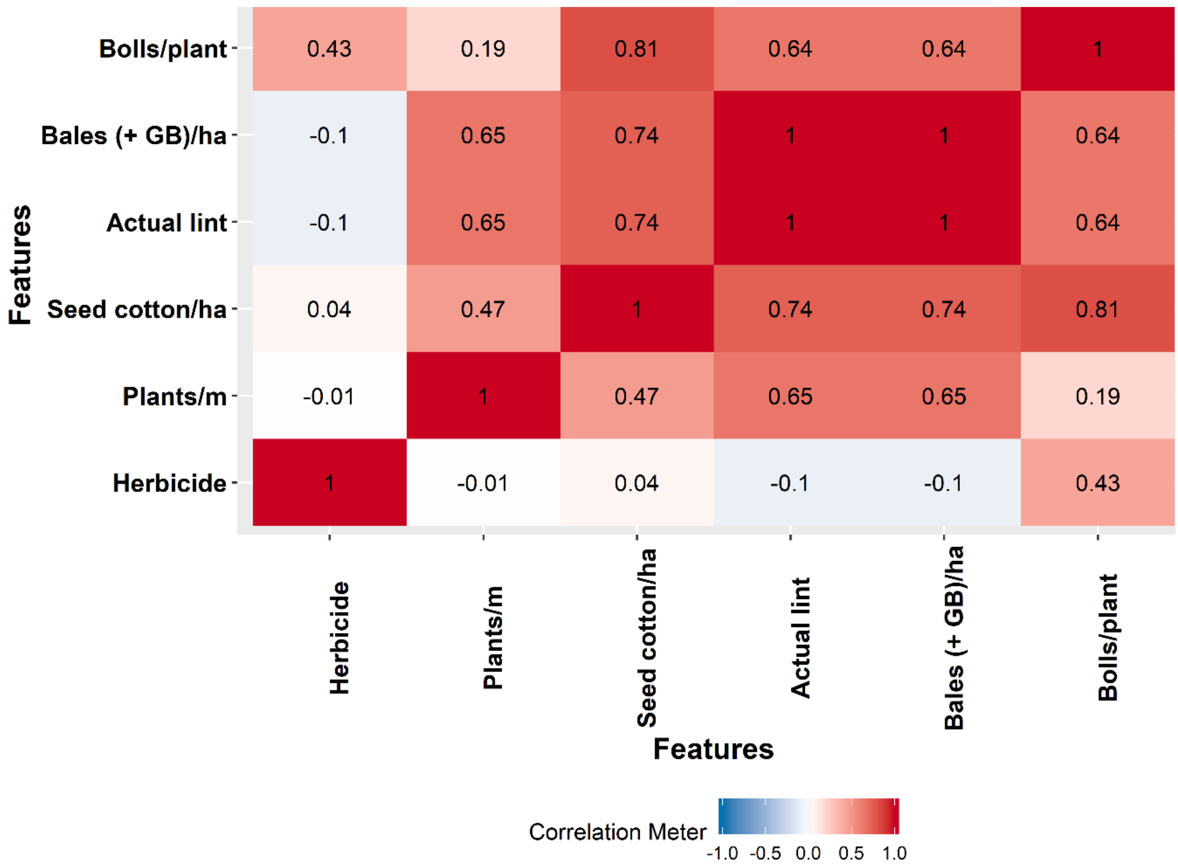


Figure 5. Correlation among yield attributes of cotton under different herbicide treatments.

Dryland system (CSD farm, Narrabri)

At Cotton Seed Distributors, Wee Waa

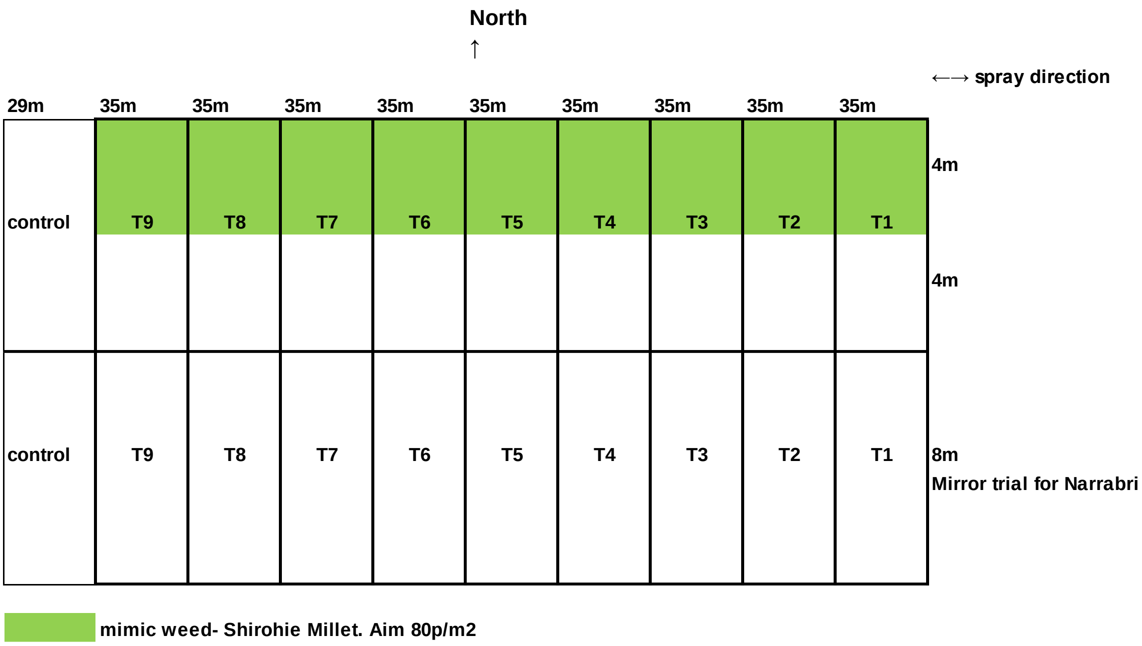
The treatment with the lowest weed pressure was Rifle®+ Glyphosate + Bouncer® applied as a mixture, compared to the weediest treatment of Glyphosate alone.

Materials and methods

The treatments were directed to some extent by the Dryland research group. With tight margins around extra herbicide applications the focus was placed on pre-emergent and at plant applications of residual herbicides + - grass mimic weeds. There is an option to look at targeted tillage or optical spraying as opposed to layby sprays.

Treatments

1. Glyphosate (1.5L/ha) only system; pre plant and post emergent Over The Top (OTT) as required, **+/-** mimic weeds to show "resistant" scenario
2. Glyphosate (1.5L/ha) pre plus Gly (1.5L/ha) + Bouncer® (1L/ha) OTT post @ 4 nodes
3. Glyphosate (1.5L/ha) pre plus directed Gly (1.5L/ha) + Rifle® (2L/ha) @ 4 nodes
4. Rifle® (2.5L/ha) pre plant (PSI) plus Gly (1.5L/ha) + Bouncer® (1L/ha) OTT @ 4 nodes
5. Diuron® (1.5kg/ha) Pre plant (PSI), & Glyphosate (1.5L/ha)
6. Conventional: Rifle® (2L/ha) (PSI) and Convoy DF® 2 Kg/ha PSPE + Glyphosate (1.5L/ha) + Gesagard® 2.5 L/ha (applied as layby)
7. Glyphosate (1.5 L/ha) only system pre plant
8. Convoy DF® (2 Kg/ha) at plant (PSI) + Glyphosate
9. Rifle® (2.5L/ha) pre plant (PSI)+ Glyphosate & Bouncer® early post emergent OTT + - cultivation @60% open boll (could also be targeted Optical Sprayer)



notes

hand sprayed with solo hand sprayer, 1.6m boom. Was quite windy. Sprayed on 2m centres
Incorporated treatments done with Lilliston cultivator
All trts had tyre rollers before planting
Millet spread with hand spinner before spraying approx 80g per trt
sown that day and planned water next day (rained next day anyway)

Field preparation and initial glyphosate application: The field was irrigated on 17th September 2018 and cultivation was done with Lilliston cultivator on 27th September. The initial glyphosate (glyphosate 540) @ 2L/ha was applied on 25th September.
Treatment placement and spray condition: All herbicide treatments were placed after sprayed the mimic weed seeds (aim 80 plants/m^2) on 3rd October 2018. The herbicides were hand sprayed with 1.6 m long boom by solo hand sprayer. The walking speed was 5.3 km/hr (expected 5.5 km/hr). Wind speed and average temperature were 14-16 km/hr (avg) and 29 ^{o}C respectively.
Cultivar and seeds sowing: Bollgard3 (cv. 746B3F) and seeds were sown after put down herbicide treatments.

Results

Weediness: The visual weediness score analysis for CSD station, Narrabri showed that the lowest weed infestation was recorded under the treatment of *Convoy DF® (2 Kg/ha) as pre + Glyphosate* followed by *Rifle® (Pendimethalin, 2.5L/ha) pre + Glyphosate & Bouncer® post.* The weed spectrum at this site was dominated by broadleaf weeds, the pre-emergent grass herbicides did little to control initial weed flushes.

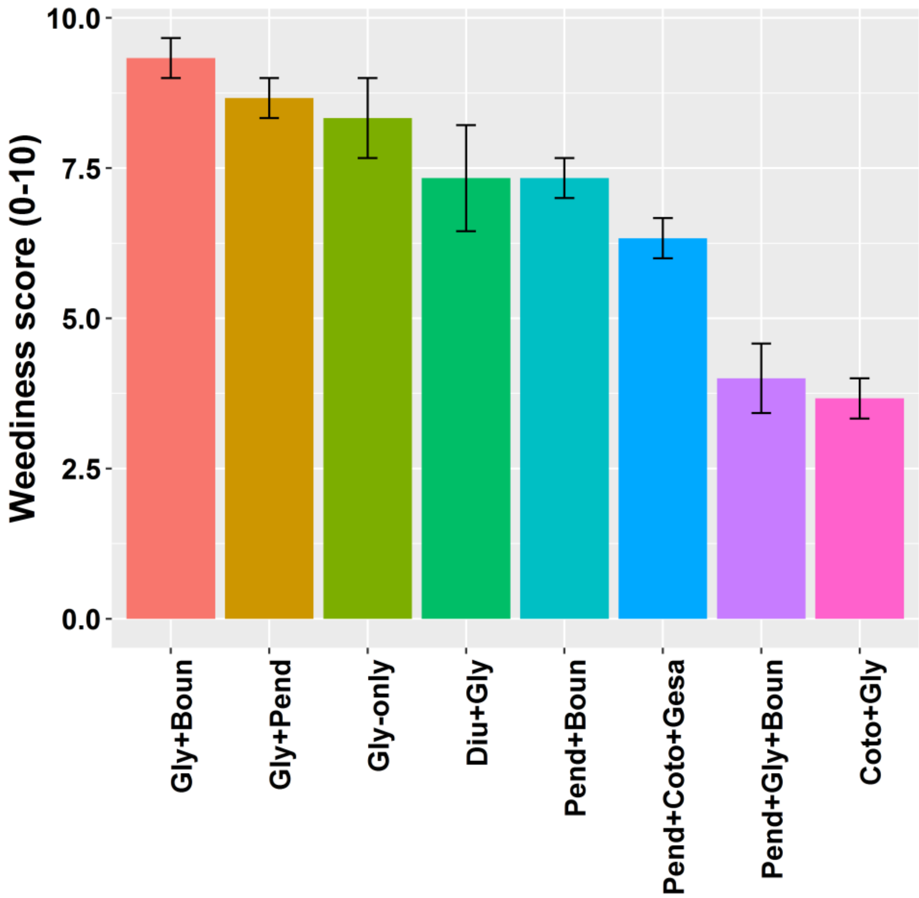


Figure 6. Initial weediness score (1-10) at CSD site. Bar represent the mean weediness score (±SE)

Toward more understanding of emerging Rosinweed *(Cressa cretica):* preliminary study

Key findings

- Growers and agronomists report that Cultivation doesn't control this species
- The species is more prevalent on sodic soils
- Chemical analysis shows that the species does not contain cyanide (with exogenous + endogenous enzymes), and toxic nitrate. Overall the risk rating for toxicity is low if stock graze this weed.

Figure. 1 A heavily infested paddock in Forbes

Outcomes

Describe how the project's outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.

The herbicide demonstration trials conducted in 2018–19 were useful extension tools which showed growers the importance of not relying on a single herbicide for weed control. This work will be built on in the new integrated weed management project across three regions within the cotton industry. Developing a consistent message and extending this to industry is an important component of the Cotton Herbicide Resistance Management Strategy (HRMS).

Studies were conducted on a number of emerging weeds identified during our surveys and listed as part of the ICAN Weeds Masterclasses in 2016. A greater understanding of the biology and ecology of these weeds will help focus future research and develop control tactics.

The identification of two populations of tall fleabane that has resistance to the glyphosate-paraquat double knock is a concern for industry and needs extending to both the cotton and broadacre farming communities.

Please describe any:-
technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);
other information developed from research (eg discoveries in methodology, equipment design, etc.); and
required changes to the Intellectual Property register.

Conclusion

Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?

The research conducted in this project supports the changes made to the Herbicide Resistance Management Strategy. Including pre-emergent and residual herbicides in an integrated program reduces the exposure of weed populations to glyphosate and as such slows the development of glyphosate resistance in our problem weeds. The biology work conducted within this project is important work essential to providing a deeper understanding of the phenology and physiology of emerging weeds in our farming system. The ability to identify a weak link in the lifecycle of weeds provides growers with opportunities to target control tactics where they will be most effective. It is about reducing the soil seedbank and running the weed numbers down so that during spray operations we are targeting small weed numbers. Regular weed surveys and screening of populations for resistance to glyphosate is important work enabling the industry to monitor changes to resistance levels and respond in real time.

Extension Opportunities

Detail a plan for the activities or other steps that may be taken:

to further develop or to exploit the project technology.

for the future presentation and dissemination of the project outcomes.

for future research.

A continuing output of weed management content through CottonInfo video is planned as well as articles in industry publications such as Spotlight. Two more scientific papers are expected to be published in the coming months. Grower field walks and industry grower meetings are on the calendar for the coming season to extend the results and inform growers of the outcomes from this project. A new weed management project with core sites in Southern and Northern NSW plus Queensland is underway with strong industry linkages and cooperation with grower groups.

9. A. List the publications arising from the research project and/or a publication plan.

(NB: Where possible, please provide a copy of any publication/s)

Journal Publications

1. Md Asaduzzman, Eric Koetz, Hanwen Wu & Adam Shepherd. 2019. Paraquat hormesis and first resistance evidence in *Conyza sumatrensis* in Australia. Crop Protection [under review].

2. Md Asaduzzaman & Eric Koetz. 2019. Germination ecology of dwarf amaranth- an emerging weed in Australian cotton system. Weed Science [under internal review].
3. Md Asaduzzman, Eric Koetz, Hanwen Wu & Adam Shepherd. 2019. First evidence double knock resistance in *Conyza sumatrensis* in Australia [Under preparation].

4. Md Asaduzzman, Eric Koetz & Hanwen Wu. 2019. First glyphosate resistant *Conyza sumatrensis* population is in Australian cotton system [Under preparation].

5. Md Asaduzzman & Eric Koetz. 2019. Fate of glyphosate resistant barnyard grass (*E. colona*) tested to 3 fops (Aryloxyphenoxypropionates) and one dim

(Cyclohexanediones) population: toward more understanding of glyphosate resistance [under preparation].

6. Koetz E.A., and Md Asaduzzaman. Susceptibility of fleabane (Conyza banariensis) biotypes to glyphosate in northern cotton farming systems of Australia. 2020 (In Press). Journal of Research in Weed Science. Vol3. Issue 2. Pp 133-144

Conference abstract

1. Md Asaduzzman & Eric Koetz. Fate of glyphosate resistant barnyard (E.*colona*) population in the presence and absence of glyphosate resistance. Cotton Research Conference, Armidale, 2019.
2. E.A.Koetz and Md.Asaduzzaman. Herbicide resistant weeds, are they winning the battle? What are the surveys telling us? Cotton Research Conference, Armidale 2019.

Presentations

Southern NSW Cotton Research update, August 14, 2019

GRDC, Northern Weeds update, Toowoomba, September 9, 2019. 'Herbicide resistance in cotton'.

Industry publications

'Southern weeds trials show no residual damage'. Spotlight, Spring 2019.

IREC Farmers Newsletter, No 202. 'Herbicide demonstration, 2018/19'.

B. Have you developed any online resources and what is the website address?

CottonInfo video series. 'Herbicide research trials 2018–19'

CottonInfo video series 'Late season weeds'

WEEDsmart, August 23 & September 16, 2019. 'No water, just weeds? Managing weeds in irrigation channels'

CRDC e-news, August 2019. 'No water, just weeds? Managing weeds in irrigation channels'

Part 4 – Final Report Executive Summary

Provide a one-page summary of your research that is not commercial in confidence, and that can be published on the internet. Explain the main outcomes of the research and provide contact details for *more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.*

This project is a 15 month follow-on from DAN1402 and continues research into herbicide resistant weeds and to identify barriers to adoption of integrated weed management in cotton, particularly the reduced use of residual and layby herbicides. Two Herbicide demonstration sites were established, one at CSD farms (Wee Waa) and the other at the Irrigation Research and Extension Committee [IREC] (Whitton). The addition of pre-emergent or residual herbicides to glyphosate resulted in lower weed numbers and higher yields in those treatments. Glyphosate alone treatments had lower lint yields than the integrated herbicide approach. A weedy control included at IREC, highlights the importance of the "Critical Weed Free Period" in cotton; allowing the weeds to grow unimpeded for six weeks reduced crop yield significantly. This research supports recent scientific publications from Graham Charles.

The project has also reported on the first case of glyphosate and paraquat resistance in two tall fleabane populations in a double knock scenario. In addition to this finding, a hormesis effect was detected when lower doses of paraquat were applied with increases in above ground biomass, plant height and buds per plant.

Weed surveys were conducted during the 2018–19 season. A smaller number of weeds were collected as a result of the dry conditions, however high levels of glyphosate resistance are still being recorded. A total of three populations (out of 11) of Sowthistle are resistant to glyphosate and two populations of Bladder ketmia (narrow) were found to be resistant to glyphosate.

A total of six ryegrass populations were screened; five populations are resistant to glyphosate and all populations are susceptible to both paraquat and 'double-knock. (glyphosate+paraquat) A total of 23, 16 and 25 populations of Barnyard grass, Windmill grass and Feathertop Rhodes grass were screened with glyphosate, and 52%, 44% and 48% populations were identified as resistant respectively.
A total of 21 and 14 populations of Feathertop Rhodes grass and Barnyard grass were treated with four Group A herbicides including three fops and one dim. Seven and five populations of Feathertop Rhodes grass and Barnyard grass showed resistance to Topik® respectively. Grass populations that are developing-resistance or are resistant to Topik® are also resistant or developing resistance to glyphosate except one population of Barnyard grass (S3) and two populations of Feathertop

Rhodes grass (D1 and D12). The importance of not relying on any one herbicide for control of problem weeds is critical for ongoing efficacy of current herbicides. Ecology and biology studies were conducted on emerging weeds collected during the weed surveys. By identifying the patterns of growth and development of these weeds, strategies for their control can be directed at weak links in their lifecycle. Dwarf, Green and Redroot amaranth do not show any seed dormancy. Temperature has significant influences on seed germination of the three tested amaranth weed species. All three species can germinate at a wide range pH solution however; they prefer to geminate in neutral to alkaline conditions. All three species are very sensitive to light and they are photoblastic. Dwarf, Green and Redroot are very sensitive to water stress but Green amaranth is more tolerant than the other two species.

In two populations of Barnyard grass, one Resistant to glyphosate, the other Susceptible, our findings found that herbicide-resistant plants of Barnyard grass will be less fit than wild type in the absence of herbicide (glyphosate), and in the absence of glyphosate, backward selection can occur. This means that without glyphosate applications, the susceptible plants of Barnyard grass, as the fittest population, may eventually dominate in the field. While resistant plants of the same population may dominate when glyphosate is used for control, this depends on glyphosate doses. A research question for the future; is this a possible non herbicide mechanism for controlling a resistant Barnyard grass population? In other words will the fitness penalty be enough to wrest control of resistant populations back or is this an additional tactic to include in an integrated weed control program.