



FINAL REPORT 2018

For Public Release

Part 1 - Summary Details

Please use your TAB key to complete Parts 1 & 2.

CRDC Project Number: DAN1402

Project Title: PostDoc. Hard to control weeds in the northern cotton farming system

Project Commencement Date:	01/07/2013	Project	Completion	Date:
	30/06/2018			
CRDC Research Program:	1 Farmers			

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Date Submitted: _____

Part 3 – Final Report

(The points below are to be used as a guideline when completing your final report.)

Background

1. Outline the background to the project.

This project built on many years of weeds work supported by CRDC and GRDC and aimed to value add to the earlier work, while providing strategic information to growers in support of MyBMP and improving information on hard to control weeds and herbicide resistant weeds.

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 remain research gaps yet to be answered. The increasing number of glyphosate resistant weed species requires an integrated approach for control driven by the updated HRMS. There is still a significant research gap around pre-emergent and residual herbicides which poses a risk to long term production and sustainability of cotton farming systems.

Weeds are a significant threat to all farming systems in NSW. Glyphosate tolerant cotton has been rapidly adopted by the Australian cotton industry since its introduction 18 years ago and currently accounts for about 99% of all cotton crops sown. This has led to a change in weed management practices with growers moving away from applying residual herbicides in anticipation of a weed problem, to dealing with known weed issues in fields using predominantly glyphosate to control surviving weeds.

These changes have resulted in a shift in the weed species found across cotton growing regions. Increasingly the broadleaf weeds: flax-leaf fleabane and sow thistle, dominate weed spectrums in cotton crops, and with increasing weed burdens in the non-cotton component of the rotation. Other important weeds include: the emerging threat of awnless barnyard grass and increasing problems with feathertop Rhodes grass and windmill grass. This project undertook a number of weed surveys to get a baseline measure of the level of glyphosate resistance in hard to control weeds of cotton farming systems.

NSW DPI has developed a program to co-invest in strategic research positions in partnership with industry. As part of this investment NSW DPI has identified key areas of expertise that are lacking and has committed to pay the salary for the Weeds Research Agronomist and also the overhead and on-costs associated with the position. It is envisaged that if industry continues to support these positions then NSW DPI will also continue to invest in these strategic positions. Weed science in northern farming systems has been identified as a key area for NSW DPI to co-invest. The appointment of an additional professional officer to conduct phenology and physiology experiments has increased weed research capacity in cotton farming systems.

Objectives

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

This project aimed to develop increased weeds research capacity within the cotton industry and improve the knowledge and understanding of critical areas including:

- The current herbicide resistance status of weeds in the cotton system including awnless barnyard grass, feathertop Rhodes grass, windmill grass, fleabane and sowthistle.
- The impact of tillage operations for pupae busting on weed control in cotton systems
- Controlled environment studies to better understand the role of temperature, rainfall, growth stage and different populations on the survival of important weeds, especially awnless barnyard grass.

The project also incorporated the role of Technical Lead for Weed management within the CottonInfo team, Eric Koetz was appointed to this role. The project has worked closely with CottonInfo Regional Extension Officers (REO's) to deliver extension messages and communicate results from herbicide resistance testing.

The project has added information to the Cotton Pest Management Guide and Crop Production Manual, Spotlight, The Australian Cotton grower, presented trial results at the Cotton Research Conference, The Australian Cotton Conference, The Australasian Weeds Conference and has one scientific publication submitted to the journal, Weed Biology and Management and one published in Crop and Pasture Science.

Project Objective 1: Appoint weeds researcher and establish links with weed researchers in the northern region.

Milestone 1.1: Weed researcher based at Wagga Wagga

Performance indicator 1.1: Eric Koetz appointed in August 2016.

Milestone 1.2: Develop linkages and networks with weed research groups in northern cotton production region and review current literature

Performance Indicator 1.2: Eric has developed strong linkages with QDAF, University of Queensland and NSW DPI weed researchers and established a strong working relationship with industry partners, GRDC weed researchers, growers, and agronomists. Eric has attended key weed research forums and presented at industry workshops, conferences and meetings. Eric was invited as a member of the Monsanto Xtend Advisory Group which has been established to develop stewardship packages for the new dicamba resistance trait in cotton.

Milestone 1.3: Appoint Research Officer

Performance Indicator 1.3: Asad Asadazuman was appointed as a research officer on the project in February 2017.

Milestone 1.4: Work with TIMS committee to complete “Weeds, Herbicides and Traits in Australian Cotton: Situation paper”

Performance Indicator 1.4: Eric was appointed to the role of co-ordinator for the Herbicide Technical Panel, the situation paper is being updated and is in final editing with co-authors. Current data from CCA and CRDC statistics are being incorporated into the document.

Project Objective 2: Survey of weeds in cotton systems.

Milestone 2.1: Undertake regional surveys of weeds in cotton systems. Screen grass weeds to group A herbicides.

Performance Indicator 2.1: An initial survey was conducted by Dr Manalil in 2015, follow-up surveys were conducted in 2016 and 2017 seasons. Across all three seasons, 250 fields have been sampled and weeds tested for resistance to glyphosate. Grass weeds collected in 2016/17 have been tested for resistance to Group A chemistry. 2017/18 samples are awaiting screening and assessment.

Project Objective 3: Controlled environment studies.

Milestone 3.1: Undertake controlled environment studies to understand processes and drivers in hard to kill and resistant weeds. Investigating phenology, dormancy, seed burial and herbicide implied dormancy.

Performance Indicator 3.1: Glasshouse and field studies were conducted and early experiments have been analysed. Later studies are in the process of being analysed ready for compilation into a report. The seed burial study will be completed by February 2019 with last bags exhumed after 24 months burial.

Milestone 3.2: Trial report written and results presented:

Performance Indicator 3.2: Results and findings from surveys have been reported to industry meetings and updates. A final compilation of all results will be available after screening is complete from the 2017 survey (in glasshouse at present, ongoing).

Project Objective 4: Impacts of pupae busting on IWM

Milestone 4.1: Field trial Established at ACRI

Performance indicator 4.1: Pupa busting trial re-designed to include a wider range of treatments. The pupae busting experiment has been modified to reflect more closely industry practice and requires an additional year of data to extract trends from trial work to present (proposed in new IWM 12 month project).

Milestone 4.2: Field data collected and analysed.

Performance Indicator 4.2: Weed counts and harvest yields recorded. No significant differences between treatments, however this is the first year of the new treatments. There was an early difference in weed species composition prior to post emergent sprays.

Milestone 4.3: Trial report written and result presented.

Performance Indicator 4.3: Ongoing, no significant differences recorded in 2017 from new trial design, an additional 12 months of trial work has been written into the IWM project 2018/19.

Project Objective: Weeds Technical Lead

Milestone 5.1: Update weeds research for industry and contribute to CottonInfo AOP.

Performance Indicator 5.1: Eric has participated in CottonInfo planning meetings and target setting for the CottonInfo Annual Operating Plan (AOP). A detailed activity plan for REO's has been developed for the coming season. Weed casestudies are complete and have been published in Spotlight, Australian Cotton grower and on the WeedSmart web site. Weed management sections of the Cotton Pest Management Guide have been updated and the Herbicide Resistance Management Strategy (HRMS) has been revised.

Project Objective 5: IWM demonstration site, pre-emergent herbicides

Milestone 5.1: Establish IWM demonstration site with pre-emergent herbicide treatments in southern NSW.

Performance Indicator 5.1: Pre-emergent applications of Terbyne® (terbuthylazine), Stomp® (pendimethalin) and control Weedmaster DST® (glyphosate only) were applied prior to sowing at the IREC field station at Whitton, southern NSW. The control of early emerging grass weeds was evident up-to the 4 leaf stage of the cotton with herbicide performance: pendimethalin >terbuthylazine >control.

Milestone 5.2: Field day for cotton industry

Performance Indicator 5.2: A successful field day was conducted at the IREC field station on January 18 2018 with > 80 growers and consultants in attendance.

Project Objective 6: Incorporation of results into WEEDpak and journal papers

Milestone 6.1: Information developed for inclusion into WEEDpak.

Performance Indicator 6.1: Data has been analysed and is available for updating of WEEDpak module.

Milestone 6.2: Submission of journal papers on project research.

Performance Indicator 6.2: Results from project have been presented at Cotton Research Conference, September 2017, Canberra. A paper is being presented at the Australasian Weeds Conference, September 2018, Sydney and a 3MT was presented at the Australian Cotton Conference, Gold Coast, August 2018. One journal paper has been peer reviewed and submitted to the journal New Zealand Journal of Agricultural Research and Journal of Crop Science and Biotechnology.

Methods

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

Project objective 2. Survey of weeds in cotton farming systems.

Three weed surveys were conducted during the project. Each of the surveys were conducted with the assistance of the CottonInfo REO's. The first in 2014/15 sampled 135 fields from Emerald in Central Qld, to Griffith in Southern NSW. In the following two seasons 2015/16 and 2017/18 a further 49 and 68 fields respectively were sampled. Seeds from the five target weeds were collected into A4 envelopes and air-dried in a glasshouse and stored for testing. Weed seeds were spread onto germination trays filled with a mixture of sand and loam, and watered up.

For the grass weeds, herbicide resistance testing occurred at the early-mid tillering stage and for the broadleaf weeds, 6–8 leaf rosette stage. The herbicide was applied using an automated laboratory

sized cabinet sprayer with a moving boom applying a water volume of 77 L ha⁻¹ equivalent from a flat fan nozzle at 300 kPa pressure. Irrigation ceased the day prior to spraying and recommenced the day after application. The trays were arranged in a completely randomized block design with three replications.

Weed control ratings were assessed visually at 28 days after treatment (DAT) using a scale ranging from 0% (no control or injury) to 100% (complete control or plant death). Total number of surviving plants for each population was counted and converted to a percentage value at 28 DAT. After visual assessments, any surviving plants were kept for seed collection. Populations with plant survival >50% were considered “resistant”, those between 20% and 50% survival “intermediate or developing resistance” and those with plant death and necrosis >80% or plant survival less than 20% were considered as “susceptible”.

The experimental design was a completely randomized block design with three replications. A single plant per pot was considered as an experimental unit and each population had six experimental units. Data were analysed using R software ([R Core Team 2014](#)) operating in RStudio ([2017](#)). Data normality and distribution was verified by Q-Q plot. The variance equality was confirmed by Levenes’ test. A logistic regression model was used to analyse the binary variable: glm (formula = plant survived ~ population, family = binomial (link = logit)).

In 2017 grass weeds were screened to glyphosate and Group A herbicides. When seedlings were at mid-tillering stage, they were sprayed with 3 L ha⁻¹ of Roundup PowerMax (540 g L⁻¹ glyphosate). When seedlings were at mid-tillering stage, they were sprayed with four different group A herbicides (Table 1).

Table 1. Application rates of four Group A herbicides.

Treat	Chemical	Product	Rate (ml/ha)	Adjuvant/surfactant
1	-	Untreated		-
2	Clodinafop (240 g/L)	Topik	125mL	Adigor 0.5L/100L
3	Haloxifop (520 g/L)	Verdict	150mL	Uptake 0.5L/100L
4	Propaquizafop (100 g/l)	Shogun	600mL	Hasten 0.5L/100L
5	Clethodim (240g/L)	Sequence	375mL	Bonza 1L/100L

Weed control ratings were assessed visually based on death symptom at 28 days after treatment (DAT), using a scale ranging from 0% (no control or injury) to 100% (complete control or plant death) at 28 DAT. Populations with plant survival >50% after spraying with herbicides were considered “resistant” whereas plant survival about 50 % to 20 % were called “developing or intermediate resistant” and population with plant survival less than 20% considered as “susceptible”.

Project Objective 3: Controlled environment studies

*Germination biology and ecology of Button grass (*Dactyloctenium radulans*)*

Methodology

Seeds of *Dactyloctenium radulans* were collected from both northern and southern growing regions of Australia in 2017/18. The species is known to have dormant seeds and therefore 100% germination was not expected. Hence different scarification techniques, including potassium nitrate (KNO₃), gibberellic acid, absolute ethanol, sulfuric acid (H₂SO₄; concentration 98%), hot and cold water were used to test breaking dormancy. A series of different concentrations and durations of these scarification methods was applied. After optimisation of scarification, germination biology of two populations (named P-N and P-S) of *D. radulans* were examined under controlled conditions, where different environmental factors, including salinity using sodium chloride (mM), light and pH buffer were imposed on seeds to observe the *D. radulans* adaptive and persistent ability. The incubated seeds were observed at 12 hours light/dark cycle at 30/22 °C for 12 days (Table 2).

Results

Among the scarification techniques, chemical scarification with sulfuric acid broke more seed dormancy and stimulated germination most effectively, followed by seeds treated with KNO_3 (2M>1M), absolute ethanol (100%) and others. Results suggests 4–5 minutes of scarification with H_2SO_4 is required to achieve maximum (90%) germination, indicating that the species has physical dormancy.

Table 2. Effect of light on seed germination of *D. radulans* of incubated at 30/20 °C alternating day/night temperature for 10 days.

Germination trait	Population	
	P-N	P-S
Seed germination (%) at light	86.00 ± 2 ^a	72.66 ± 1.76 ^b
Seed germination (%) at dark	55.33 ± 1 ^a	21.66 ± 1.3 ^b
Relative light germination (RLG)	0.61 ^b	0.77 ^a
Germination index (GI)	73.60 ± 1.2 ^a	40.73 ± 3.8 ^b

There was a significant effect of light ($P < 0.001$) and populations ($P < 0.001$) treatments, and the interaction of both factors was significant ($P < 0.01$). Population P-N (collected from north) had at least 77% germination in light conditions (Table 2). Significantly a high proportion of seeds of both populations germinated at light/dark cycle than in total darkness. This demonstrates that both populations of *D. radulans* are photoblastic, however P-N showing higher germination in either condition than P-S (collected from south). Populations differed significantly ($P < 0.001$) in terms of relative light germination (RLG) and germination index (GI). P-S had a higher RLG than P-N.

Seed germination decreased in both populations with increasing salt concentration. Germination was greater than 20% in a concentration of sodium chloride (NaCl) of 50 mM. Germination of P-N in the 100 mM solution was still higher than P-S. The differential delay in germination according to populations, or with germination of population P-N being less affected at the highest rate of salinity overall, indicates that some adaptation to saline conditions is present in this species.

Germination of both populations was strong to very strong in all buffer solutions ranging from pH 6 to 10 (Figure 1). The germination of both populations was stimulated with higher pH values, which infers that the alkalinity of the environment is not a critical or limiting factor for *D. radulans*. The overall germination response was very strong, and each of these populations did vary significantly in their response to varying pH.

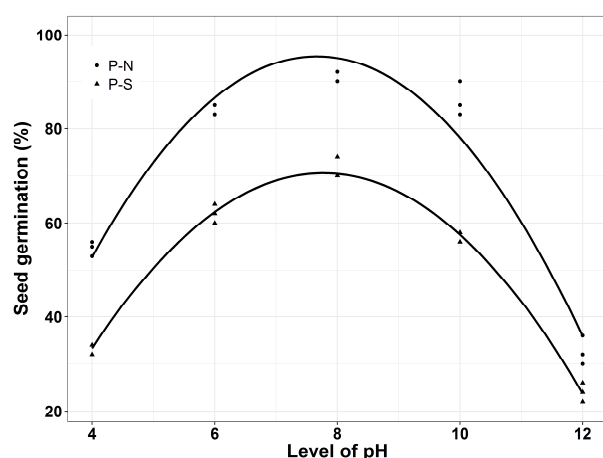


Figure 1 Effect of buffered pH solutions on germination of scarified seeds of *D. radulans* incubated at 30/20 °C alternating day/night temperature for 10 days.

Germination biology of Bladder ketmia (*Hibiscus spp.*)

Methods

Seeds of single population of both wide and narrow leaf Bladder ketmia were collected from the same cotton farm located in the southern farming system in 2017/18. The collected seeds were scarified with H_2SO_4 for 20 minutes and placed on petri dishes under controlled condition to observe the inter-specific variation of germination biology of this species in response to environmental factors. Another portion of scarified seeds were placed on plastic pots pre-filled with commercial potting mix. Growing plants in pots were regularly irrigated and glyphosate at four different rates (0, 0.8, 0.75 and 1.5 L/ha) were sprayed at pod development stage. The sprayed plants were assessed visually after 28 days of glyphosate application and seeds were collected from sprayed plants.

Results

Wide leaf ketmia was twice as tolerant to salt than narrow leaf ketmia and at 100 mM NaCl, about 70% of the seeds of wide leaf ketmia are still able to germinate (Figure 2). Narrow leaf ketmia is more sensitive to water stress and its germination was completely inhibited at -2 Mpa water potential (data not shown). Glyphosate spray at pod development stage demonstrates, narrow leaf ketmia is 3 fold more tolerant to glyphosate than wide leaf ketmia and 1.5 L/ha glyphosate is required to kill 50% individuals of tested population of narrow leaf ketmia (Figure 3). Resistant plants of narrow leaf ketmia produced seeds under all glyphosate rates, however viable seeds were only produced at glyphosate rates up to 0.75 L/ha. More research through resistance surveys and fitness to glyphosate system of these two species is needed.

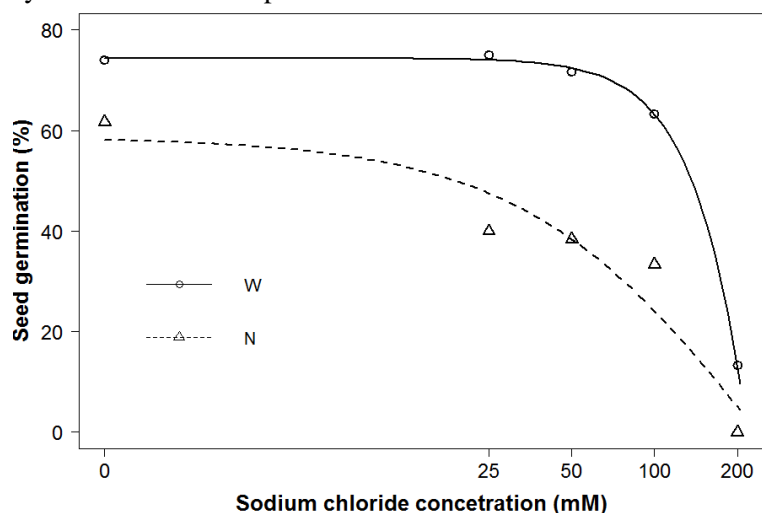


Figure 2. Effect of sodium chloride on germination of scarified seeds of Bladder ketmia incubated at 30/20 °C alternating day/night temperature for 10 days.

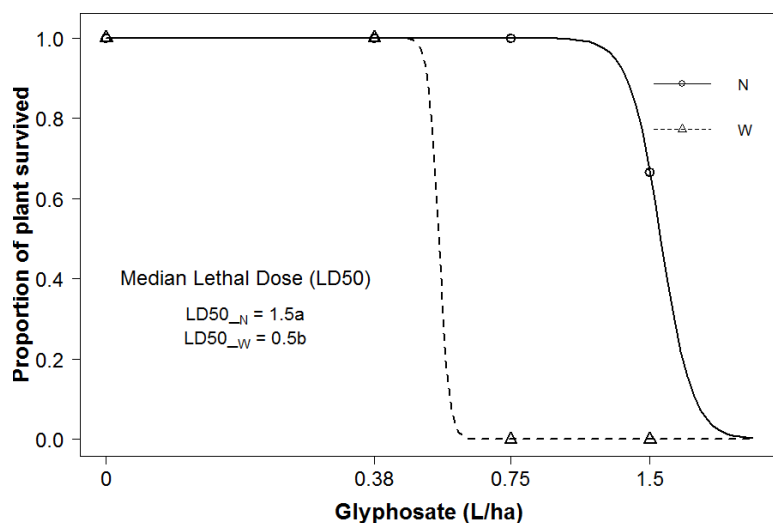


Figure 3. Response of wide and narrow ketmia to different levels of glyphosate sprayed at pod development stage.

Early interference between Cotton and Bladder ketmia (*Hibiscus trionum* var. *trionum*) seedlings

Summary

- At seedling stage (Cotton: Narrow leaf ketmia = 25%: 75% ratio), narrow leaf ketmia can also significantly reduce root biomass of cotton.
- This crop-weed ratio can cause the delay for first square setting in cotton plant.

Methods

Early weed and cotton interference was examined under glass house conditions in 2017/18. The seeds of cotton and narrow leaf ketmia were sown simultaneously in 9 cm width pots prefilled with field soil. Before sowing, seeds of the weed species were scarified with H₂SO₄ for 20 minutes to minimise dormancy issues. Bladder ketmia was chosen here because it commonly coexists in cotton and represents important similarity in physiology, morphology, and canopy architecture that may contribute to differences in competitive ability. In this replacement series experiment, each species was paired with other. Each series consisted of monocultures and three mixtures at 75:25, 50:50, and 25:75 ratios. The plant height, root biomass, above ground biomass of both species was measured. The position of first square of cotton was also assessed just before harvest of the plant.

Results

Plant height and above ground biomass of cotton was not affected by ketmia density (regardless of intra species interference) however root biomass of cotton significantly reduced at 25:75 cotton: weed pairs (Figure 4). Under same crop-weed pairs, the first square's development was delayed in cotton (Table 3). These results suggest that the interaction among the cotton and ketmia pairs was competitive or allelopathic or both rather than mutually stimulatory. This study was performed in a relatively stable and productive climate; moreover, water and nutrient levels were always adequate. Under natural conditions, or in other climates, different plant attributes may contribute to the competitive abilities observed here. Further studies relating specific environmental or management impacts to the growth and competitiveness of plants may provide the basis for implementing weed control strategies that rely on monitoring and manipulation of species composition

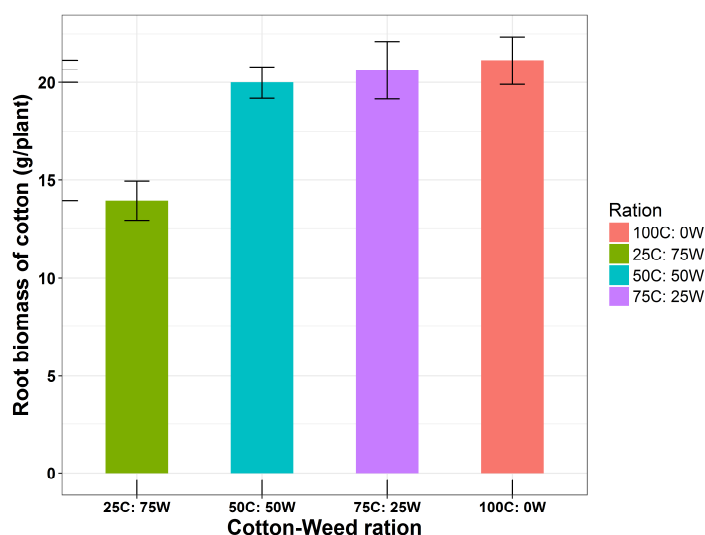


Figure 4. Mean root biomass (g/plant) of cotton at four different cotton-narrow leaf ketmia pairs.

Table 3. Influence of ketmia and cotton weed pairs on position of first square

Cotton: Weed	Position of first square after node's number
100:0	6.62a
75:25	6.62a
50:50	6.68a
25:75	6.93b

Results

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

In 2014/15 weeds collected from 134 fields were screened for glyphosate resistance. The resistance levels for each species are summarised in Table 4.

Table 4. Weed species collected in 2015, % glyphosate resistant

Weed species	Collected samples	Resistant %	Developing resistance %	Susceptible %
Sowthistle	37	16	6	78
Fleabane	37	92	5	3
Feathertop Rhodes grass	20	10	10	80
Windmill grass	24	45	45	5
Barnyard grass	24	22	50	28

The 2016–17 results are listed below.

Fleabane

Out of 25 fleabane populations in 2016/17, 19 (>75%) are resistant to glyphosate, where, seven populations are categorised as resistant and 12 populations as developing/intermediate resistant. The remaining six populations did not show any resistant symptoms (Figure 5). When fleabane seedlings were at the rosette stage (8–10 cm diameter, 8–10 expanded leaves), they were sprayed with 0.65 kg ae ha⁻¹ of glyphosate, which is a commonly used rate for general fallow weed control in Australia (Walker *et al.* 2011).

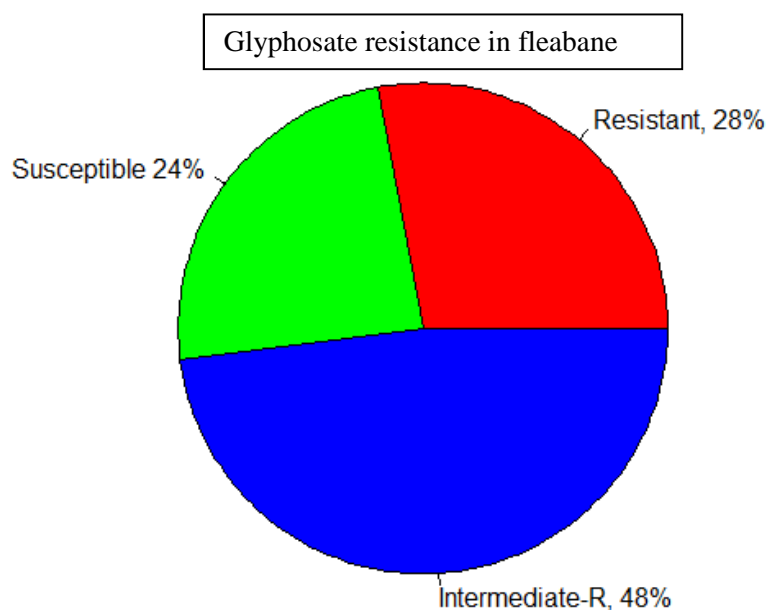


Figure 5. Twenty five fleabane populations collected and screened against glyphosate in 2017. The tested populations are categorised as resistant (28%), intermediate resistant (48%) and susceptible (24%) based on plant survived at 28 days after treatment.

Barnyard grass

Out of eight populations of barnyard grass, five populations are resistant to glyphosate and three populations are categorised as susceptible (Figure 6)

Glyphosate Resistant in Barnyard grass populations

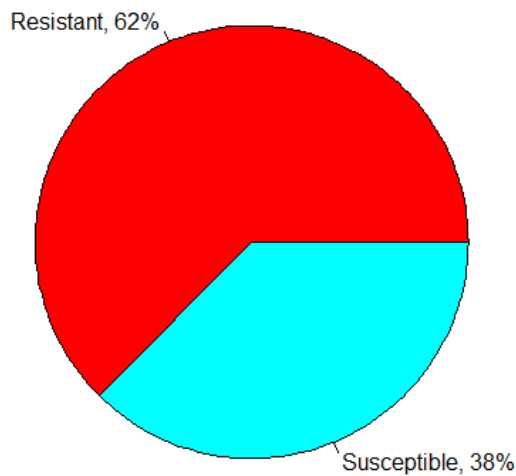


Figure 6. Eight Barnyard grass (*Echinochloa. colona*) populations were screened against glyphosate in November 2017.

Feathertop Rhodes grass

Only two populations of feathertop Rhodes grass are resistant to glyphosate, and two populations are categorised as developing/intermediate resistance. The remaining eight populations did not show any resistant symptoms). The significant differences between resistant and susceptible populations are further distinguished from the mean difference or comparison analysis (Figure. 7).

Glyphosate Resistant in Feathertop Rhodes grass populations

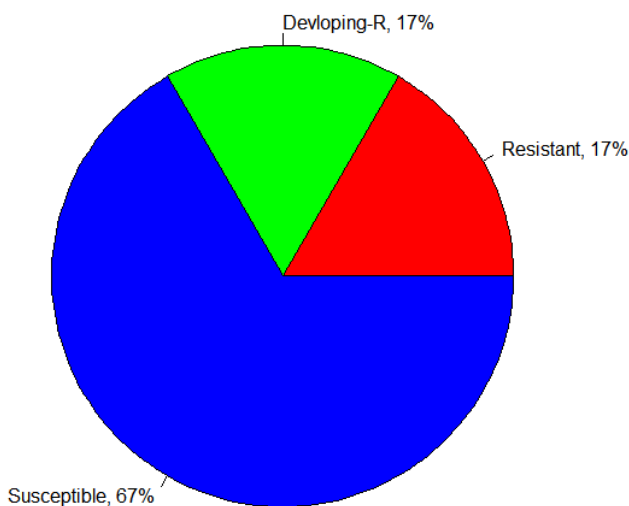


Figure 7. Twelve feathertop Rhodes grass populations were screened against glyphosate in November 2017.

Windmill grass

Visual scoring on plant damage revealed that about 45% populations of Windmill grass were resistant to 3 L ha⁻¹ of Roundup PowerMax® (540 g/L glyphosate) (Figure. 8). The most resistant populations were N1 and N2. More than 50% populations were susceptible to glyphosate.

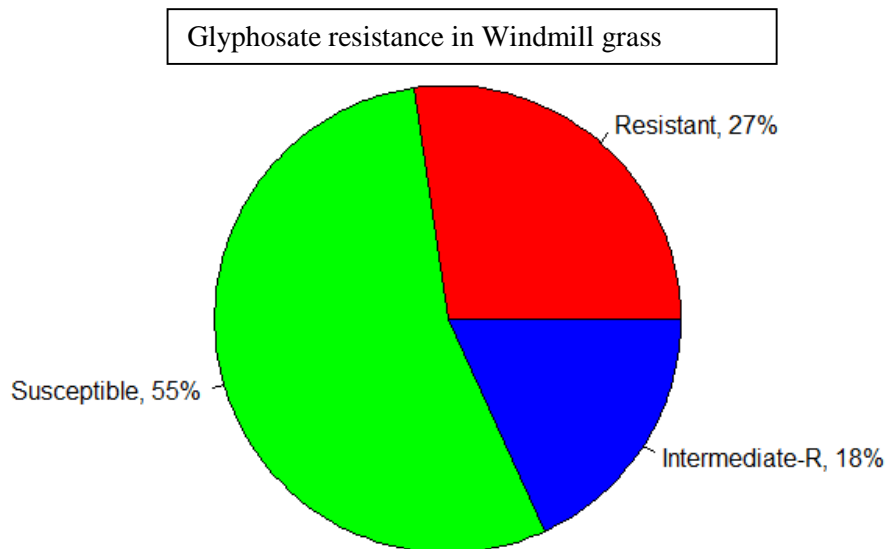


Figure 8. Eleven Windmill grass populations were screened against glyphosate in 2017.

Sowthistle

In 2015, 37 samples of sowthistle were collected and screened to glyphosate. When sowthistle seedlings were at the rosette stage (8–10 cm diameter, 8-10 expanded leaves), they were sprayed with 0.65 kg ae ha⁻¹ of glyphosate. Six populations were classified as resistant, two developing resistance and 29 susceptible (Figure 9). This was a pleasing finding considering high levels of resistance in other species tested.

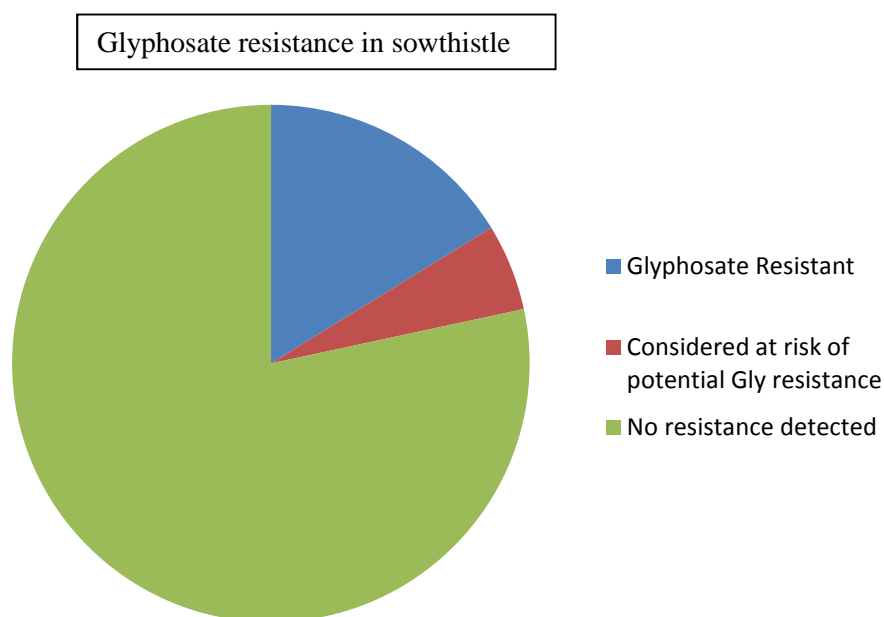


Figure 9. Thirty seven samples of sowthistle collected in 2014/15 screened for glyphosate resistance.

Group A herbicide screening.

Visual scoring on plant morphological damage revealed that among the group A herbicides, Verdict™ and Sequence® are the most effective to control tested grass populations. On the contrary, two populations (25%) namely BG11 and H4 showed resistant to Shogun®. Population H4 also showed resistance to Topik® (Table 5). The remaining populations are susceptible to all applied group A herbicides.

Table 5. Plant (%) survived after application of four group A herbicides on 8 different populations of Barnyard grass (*E. colona*)

SL no	Population ID	GPS E S	*Plant survived (%)			
			Shogun	Topik	Verdict	Sequence
1	BG1	27 32 75 151 35.996	0	0	0	0
2	BG11	28 090 06 14 843 339	100 R	0	0	0
3	M1	29 24925 149 583 84	0	0	0	0
4	BG14	28 314 72 150 020 06	0	0	0	0
5	W3	31 475 93 150 020 06	0	0	0	0
6	BG8	26 543 07 150 581 84	0	0	0	0
7	H4	26 543 82 150 595 08	100 R	100 R	0	0
8	BG2	27 294 03 151 299 897	0	0	0	0

*R= Resistant

Plants of two populations (17%) of Feathertop Rhodes grass were able to survive under the toxic effect of both Shogun® and Topik®, these populations are FT6 and FT8 (Table 6).

Table 6. Plant survived (%) after application of four A herbicides on 12 different populations of feathertop Rhodes grass

SL no	Population ID	GPS E S	*Plant survived (%)			
			Shogun	Topik	Verdict	Sequence
1	FT14		0	0	0	0
2	M5		0	0	0	0
3	FT6		50 DR	100 R	0	0
4	FT13		0	0	0	0
5	FT12		0	0	0	0
6	FT3		0	0	0	0
7	FT5		0	0	0	0
8	FT7		0	0	0	0
9	FT8		70 R	100 R	0	0
10	Kar**		0	0	0	0
11	Sample 1		0	0	0	0
12	Sample 2		0	0	0	0

*R= Resistant; DR=Developing-R

Visual scoring on plant morphological damage revealed that among the tested group A herbicides, Shogun is most effective to control Windmill grass population including previously identified three glyphosate resistant populations. Topik® was less effective and 100% tested populations were resistant to this herbicide (Table 7). Three populations namely N1, N2, and IWM38 showed resistance to Verdict™ Two glyphosate resistant populations collected in 2015/16, expressed an intermediate resistant to Clethodim at 240g/L.

Table 7. Plant survival (%) after application of four different group A herbicides on 11 populations of Windmill grass

SL no	Population ID	GPS E S	*Plant survived (%) and R, IR or S			
			Shogun	Topik	Verdict	Sequence
1	N1	30 108 7 149 43591	0	100 R	33 IR	0
2	N2		0	100 R	33 IR	0
3	W1	31 733 92 147 377 49	0	100 R	0 S	0
4	W4	31 502 69 148 016 2	0	100 R	0 S	0
5	W6	32 144 08 147 496 64	0	100 R	0 S	0
6	IWM34	30 124 905 149 318 04	0	100 R	0 S	33 IR
7	IWM36	30 141 34 149 362 650	0	100 R	0 S	33 IR
8	IWM 38	31 496 56 147 425 653	0	100 R	55 R	0
9	H6	33 260 45 145 340 58	0	67 R	0 S	0
10	M4	29 232 59 149 462 05	0	50 IR	0 S	0
11	N3	30 280 06 149 574 46	0	50 IR	0 S	0

*R= Resistant, IR=Intermediate/Developing Resistant and S= Susceptible

Outcomes

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

Objective 1: Appoint Weed researcher and establish links with northern weed researchers.

Eric Koetz was appointed to the Weed Research role in August 2016 based at the Wagga Wagga Agricultural Institute. Since his appointment Eric has been active in attending Industry meetings and research forums and developed networks amongst weed researchers in the northern region. Eric has worked closely with CottonInfo REO's to develop weed survey protocols for the collection of weed seeds for herbicide resistance testing focussing on glyphosate. Eric has attended WeedSmart workshops, Crop Consultants Australia (CCA) workshops and annual conferences and Cotton Grower Association (CGA) meetings. Asad Asadazuman was appointed as a Research officer in February 2017 to assist with glasshouse screening and weed biology experiments. The situation paper has been updated with recent herbicide resistance data and trait information and is in the final stages with co-authors for review.

Objective 2: Survey of weeds in cotton systems.

An initial survey conducted in 2014/15 by Dr Manalil sampled 135 fields collecting fleabane n=(37), sowthistle (37), windmill grass (9), feathertop Rhodes grass (11) and awnless barnyard grass (18). In 2016/17 an additional 49 fields were sampled with fleabane (31), sowthistle (6), windmill grass (11), feathertop Rhodes grass (9) and awnless barnyard grass (7) collected. A final survey was conducted in 2017/18 (68 fields) with a mixture of random sampling and targeted growers identified by REO's. In addition to the five major weeds identified within the project bladder ketmia and button grass seed was collected from several locations. Fleabane (8), sowthistle (20), windmill grass (19), feathertop Rhodes grass (26) and awnless barnyard grass (21) were collected and are in initial stages of preparation for testing.

From the original survey in 2015 very high levels of glyphosate resistance were detected amongst the selected weeds.

- Fleabane >95% R
- Windmill grass 95% R
- Awnless barnyard grass 72% R
- Sowthistle 22% R
- Feathertop Rhodes grass 20%

Results from the 2016 survey also showed high levels of resistance from the samples collected.

- Fleabane >75% R
- Windmill grass 65% R
- Awnless barnyard grass 60% R
- Sowthistle 10% R
- Feathertop Rhodes grass 35%

Whilst there has been some variation around the levels of resistance detected, there are consistently high levels of resistance, which indicates a reduction in glyphosate efficacy in common weeds of cotton systems.

In addition to glyphosate testing, grass weeds were screened against a range of common Group A herbicides from samples collected in 2016.

- The efficacy of four group A herbicides are different in terms of Windmill grass control at mid-tillering stage and the effectiveness order is: Shogun®>Verdict™>Sequence®>Topik®.
- All tested (100%) populations of Windmill grass are resistant to Topik®
- Some populations of Windmill grass are resistant to both glyphosate and group A herbicide.
- One population of Nut grass and 1 population of Silver grass showed developed and intermediate resistant to glyphosate @ 3 L ha⁻¹ at mid-tillering stage respectively.
- Overall, Verdict™ and Sequence® are more effective than Topik® and Shogun® to control all tested grass populations.
- Two populations of Barnyard grass, two populations of Feathertop Rhodes grass and one population of Silver grass expressed resistance to Shogun®.
- Two populations of feathertop Rhodes grass, one population of Barnyard grass, Liverseed and Silver grass are resistant to Topik®.
- One population of both Barnyard grass (named BG 11) and feathertop Rhodes grass (named FT6) demonstrated resistance to both glyphosate and Shogun®.

The reporting of herbicide resistance in grass weeds to Group A chemistry is not new, however when considered with the high levels of glyphosate resistance reported, alternative control options are reduced. Additional testing for Group A resistance will be conducted on the samples collected in 2017/18. This is currently underway with germination occurring in temperature controlled rooms. They will then be prepared for testing and screening in the spray cabinet.

In collaboration with CottonInfo REO's, weed case studies were completed across all cotton valleys. Growers were interviewed and their answers compiled into narratives about their farming systems and the management of weeds. These case studies were published as an e-newsletter through the CottonInfo network and on the WeedSmart web site.

Objective 3. Controlled environment studies

Glasshouse and controlled environment studies were undertaken at Wagga in 2016/17. Dormancy and seed burial studies were conducted and the last seeds will be retrieved from the field in summer 2018.

Phenology/Fitness outcomes in glyphosate resistant Barnyard grass

A glyphosate resistant individual of barnyards grass can still survive under a high dose of glyphosate by sacrificing phenological (eg. reduced plant height, more time for vegetative stage) and reproductive features (eg. reduce number, and length of spikelets, shorter rachis, reduced total

seeds/inflorescence). These changes might be due to costs of adaption under high doses of toxic glyphosate salt.

Hormesis effects in glyphosate resistant tall fleabane

Low doses (0.25 L/h & 0.5 L/ha) of paraquat can stimulate the growth of tall fleabane and both resistant (R) and susceptible populations shows a similar initial stimulation/hormetic effects, but only R plants/populations sustained the hormetic effect through maturity. Thus, hormesis can significantly benefit R plants but not S plants/populations.

Persistence ability of Echinochloa crus-galli and Echinochloa colona (burial study)

E. crus-galli is more persistent than *E. colona* (based on 9 months' burial treatment).

Shikimate accumulation in R-and S- populations of Windmill grass

Based on absorbance reading from spectrophotometer (need to convert the absorbance into amount by slope) we can say that glyphosate resistant populations of windmill grass accumulate lower amounts of shikimate acid than susceptible populations in their leaf discs. This is further confirmation of our traditional screening results and supports results from published research by the ARHI group in WA.

Germination biology and ecology of Button grass (Dactyloctenium radulans)

- *D.radulans* shows a high level of physical seed dormancy & seedlings of this species can survive a moderate level of water stress.
- Populations from northern cotton farming system are more tolerant to salt stress than southern populations.
- Seeds can germinate over a wide range of pH (between 4 and 10) but the species prefer to germinate in alkaline soil.
- Light can heavily stimulate the germination of this species, so agronomic tactics such as tillage practice, mulching, or crop residues can play a role to minimise its infestations.

Early interference between Cotton and Bladder ketmia seedlings

At crop seedling stage (Cotton v Narrow leaf bladder ketmia = 25%: 75% ratio), narrow leaf bladder ketmia can cause the delay of first square setting (and the addition of nodes by two days) in cotton plants. This crop-weed ratio can also significantly reduce the root biomass of cotton.

Objective 4. Impacts of pupae busting on IWM

A field trial was established at ACRI in 2015. After two seasons a meeting of the project group decided to introduce new treatments into the design to “push the system”. The new treatments included a whole systems approach introducing pre-emergent and in-crop residual herbicides.

The updated field protocol is below. It is anticipated that two seasons will be required to produce weed species shifts and changes in weed populations. As a result, an additional season has been incorporated into the new 12 month IWM weed project. Graham Charles will continue to oversee day to day operations.

Field 7 Protocol

Was	New		Weed management inputs	At plant	Post resid	Early gly	Late gly
W1	W1	Pre resids	At-planting residuals only (no glyphosate)	resid			
W3	W2	Pre + Post	At-planting + post-emergence residuals (no glyphosate)	resid	post		
W3	W3	Gly only	Glyphosate ONLY			gly	gly
W1	W4	At-resid + gly	At-planting residuals + early glyphosate	resid		gly	
W2	W5	Post+ gly	Post-planting residuals + glyphosate		post	gly	gly
W2	W6	All	At-planting residuals + glyphosate+chipping	resid		gly	gly

Post harvest tillage Management-		
T1	Nil	Control (no cultivation)
T2	Early-Lil	Early Low tillage (Lilliston)
T3	Early-Gd	Early Medium tillage (Go-devil)
T4	Late-Lil	Late Low tillage (Lilliston)
T5	Late-Gd	Late Medium tillage (Go-devil)
T6	Cent bust	Late Centre Bust

Timing	Crop stage	Field operations
Late-Sept	Pre-planting	Record weed density & estimate biomass Field to be weed free at planting, use Valor or Spray.Seed to remove any weeds after observation
Early-Oct	Pre-planting	Pre-irrigate
Mid-Oct	At-planting	Apply residuals - treats 1, 2, 4 & 6 Flush experiment
Late-Oct	At emergence	Record crop emergence
Mid-Nov	3 weeks post-emergence	Record weed density & estimate biomass
Late-Nov	4 weeks post-emergence	Apply 1 st glyphosate – treats 4, 5 & 6 Apply residuals – treats 2 & 5
Early-Dec		Clean out pathways
Late-Dec		Apply 2 nd glyphosate – treats 5 & 6
Mid-Jan		Record weed density & estimate biomass
Late-Jan		Apply 3 rd glyphosate – treats 5 & 6 Apply residuals – treats 2 & 5
April	Defoliation	Defoliant to all
May	Picking	Pick all
Late-May	Post-picking	Record weed density & estimate biomass
June	Tillage	Cultivate early tillage treatments
August	Tillage	Cultivate late tillage treatments

Objective. Weeds Technical Lead.

Eric Koetz was appointed as the Technical Lead for Weed Management in February 2017. Eric has participated in CottonInfo planning meetings and developed the weed management tasks for REO's AOP to undertake as part of their work plan. Eric has assumed the role as the co-ordinator of the Herbicide Technical Panel and has reviewed and updated the weed management sections of the 2018 Australian Cotton Production Manual and the 2018 Cotton Pest Management Guide. As part of the review the HRMS has been updated following consultation with Weed researchers and the TIMS panel. Eric participated in the 2017 study tour to the US looking at the introduction of dicamba and 2,4-D tolerant cotton. The tour was an opportunity for industry representatives to see firsthand off target damage and also the efficacy of the new technologies in combating serious herbicide resistant weeds.

Objective 5. IWM demonstration site with pre-emergent herbicides.

A demonstration trial was established at the IREC field station at Whitton in 2017. On October 14, 2017 demonstration plots were sown at the IREC field station. Three treatments, a control plot with glyphosate early post, Terbyne® (1kg/ha) post sow pre-emergent and Stomp® (3L/ha) post sow pre-emergent were applied. On 1st November weed establishment counts were conducted using a 50cm X 50cm quadrat, randomly distributed across the plots. The dominant weed species recorded were barnyard grass and annual ryegrass. Averaged across both replicates and 20 quads per plot, the treatment hierarchy was, Glyphosate had 102 plants/m², Terbyne® 57/m² and Stomp® 5/m². The trial demonstrated to growers the benefits provided from adding pre-emergent herbicides to a glyphosate system without impacting on the established plant stand of the cotton. There was no significant difference in crop establishment between herbicide treatments. The argument against using pre-emergent herbicides in the southern valleys surrounds concerns from the cooler, wetter sowing

windows. This trial at the IREC site in 2017 demonstrated that applied at the correct timing and with the right equipment, plant establishment was not compromised and weed control was improved.

Objective 6. Incorporation of results in WEEDpak and journal papers.

Key results from the survey and herbicide resistance testing will be incorporated into a WEEDpak update. There is one paper which has been submitted to the Journal, Weed Biology and Management. A presentation of results from the project was made at the 2017 Cotton Research Conference in Canberra, 5-7 September 2017. A paper on the seed dormancy and biology of Bladder Ketmia will be presented at the Australasian Weeds Conference, September 9-12 2018 in Sydney. There have been a number of articles and presentations during the course of the project, these are listed in section 9.

Please describe any:-

- a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);
- b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and
- c) 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 five weeds identified as hard to control in cotton farming systems (fleabane, sowthistle, windmill grass, awnless barnyard grass and feathertop Rhodes grass) have all had glyphosate resistance confirmed from three random surveys within this project. The levels range from a concerning 25% for feathertop Rhodes grass to serious >95% in fleabane.

Results from this project have been incorporated into changes of the HRMS and the protocols have been updated. Growers are more aware of resistance levels with results from surveys appearing in industry publications and the results for individual fields mailed out to growers. Already we are seeing a slight increase in the usage of residual and pre-emergent herbicides (CCA data 2016/17).

The engagement with the CottonInfo REO network has been pivotal to conducting the surveys and communicating results to growers. There has been an increase in the use of residual herbicides and pre-emergent chemistry (CCA data 2017), a positive sign that the message is getting through.

Extension Opportunities

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

- (a) to further develop or to exploit the project technology.

A new research project has been funded for 12 months to expand on the introduction of non-glyphosate herbicides for the control of hard to kill weeds. Four proposed demonstration sites will be established in the 2018/19 season, two dryland and two irrigated (one at Whitton and one at Wee Waa). Depending on weather conditions dryland trials may not be possible this season. The trials will focus on pre-emergent and residual herbicides and their role in controlling herbicide resistant weeds, thus reducing the selection pressure on glyphosate. This extension will also allow for another year of data to be collected from the pupae busting experiment at ACRI. These trials will be established with the co-operation of Nufarm, NSW DPI, CSD and CottonInfo.

- (b) for the future presentation and dissemination of the project outcomes.
 - Presentations will be made at the 2018 Cotton Conference and the 2018 Australasian Weeds Conference.
 - A summary of results will be presented at CCA workshops in 2018

- Articles will continue to be worked up for distribution through cotton industry media and the WeedSmart web site.
- Extension of project findings and presentation at preseason meeting, AGnVET Warren, August 23, 2018.
- One paper has been submitted to ‘Weed Biology and Management’
- Article submitted to The Australian Cotton grower.

(c) for future research.

The combination of glyphosate resistance and detection of Group A resistance in several grass populations will put strain on the long term efficacy of glyphosate. Research into pre-emergent and residual herbicides for grass weed control in non-crop as well as in-crop situations is important to obtain a clear understanding of how to manage populations of weeds resistant to multiple modes of action. In addition a clearer understanding of the role of residuals in fallow weed control is an area of future research.

The economics around the introduction of residual and pre-emergent herbicides into dryland cotton farming systems is an important question that still requires answers. Moving forward, the introduction of stacked traits into the Australian cotton farming system requires further investigation. It is important to understand how the new herbicides will be used in managing problematic hard to control weeds. The integration of this new technology into the cotton industry HRMS provides opportunities for further research, however a regional focus will be important to understand the efficacy of this new technology. Reports in the US of off target damage to other crops and ornamentals is a concern moving forward for the Australian cotton industry.

Concern surrounds the new traits in cotton, especially if off target damage occurs and the questions are asked about Agriculture’s “Social license to farm.”

Publications

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)

Presentations

- August 2016, Southern Cotton R & D Update, Yanco. Herbicide resistance surveys.
- January 2017, Toowoomba. Herbicide Technical Panel meeting, Resistance update.
- June 20, 2017. MacIntyre Valley CGA, End of season review. Herbicide resistance update.
- July 5, 2017, WeedSmart Podcast. Cotton Update
- July 27, 2017, Cotton Collective, Griffith. Cotton 101 session, weed management.
- August 22, 2017. Southern Cotton R & D Update, Yanco. Hard to control weeds, survey results.
- August 23, 2017, GRDC Northern Weeds Update, CSU Wagga Wagga. Update of cotton research and herbicide resistance surveys.
- November 2, 2017, Southern Valley CGA AGM. Presentation of observations and lessons from US weed study tour.
- IREC Field Day, January 18, 2018. Presentation: “Herbicide resistance, pre-emergent herbicides. 80 growers and consultants in attendance”.
- Presentation Herbicide Technical Panel, Narrabri 21 February 2018. Herbicide resistance results, DAN1402.
- May 22, 2018. Toowoomba. Weed management in cotton research, a review
- August 7, 2018. 3MT: Births, Deaths, Taxes and herbicide resistance. In the “War on Weeds” session. Australian Cotton Conference, Gold Coast, 2018

Publications

- Spotlight, Winter 2017. Page 13-14. Integrated Weed Management: Time to take a stand against survivors.
- Spotlight, Winter 2017. Page 14. Pupae busting and weed control
- Spotlight, Winter 2017. Page 15. What the weed surveys show.

- September 2, 2017; WeedSmart ask an Expert. How can you take the pressure off glyphosate across the cropping rotation?
- Spotlight, Spring 2017 Page 29. New CottonInfo faces in the field.
- The Australian Cotton grower. October – November 2017. Vol 38.No.5. pages 20-24. US tour highlights tough weed lessons for Aussie growers.
- CottonInfo e-news January 2018. “How do other growers control their weeds?”
- CottonInfo video series, January 2018.
 - Sources of weed seeds.
 - Minimising glyphosate resistance.
- Acres of Opportunity Video series. “IWM Integrated Weed Management”. February 2018
- Spotlight, Autumn 2018, Page19. “How can we best manage new herbicide traits in our cotton crops?”
- Spotlight, Autumn 2018, Page 20. “Resistance on the rise”

Journal papers

Manalil, S., et al. (2017). "An assessment of weed flora 14 years after the introduction of glyphosate-tolerant cotton in Australia." *Crop and Pasture Science* **68**(8).

Koetz, E.A., M.D Asaduzzaman. (2018). Susceptibility of fleabane (*Conyza bonariensis*) biotypes to glyphosate in northern cotton farming systems of Australia. (Submitted to New Zealand Journal of Agricultural Research and Journal of Crop Science and Biotechnology).

Conference Papers

Koetz, E.A., M.D Asaduzzaman. (2017). Hard to control weeds in northern cotton farming systems. Cotton Research Conference, September 7, 2017.

Asaduzzaman, M.D, E.A. Koetz (2018). Inter-specific variation in seed dormancy and germination biology of bladder ketmia (*Hibiscus spp.*) 21st Australasian Weeds Conference, Sept 2018.

Industry Publications

Koetz, E.A., M.D Asaduzzaman. (2017). Investigating the current herbicide status of problem weeds in northern cotton farming systems. NSW DPI Southern NSW research results 2017.

Asaduzzaman, M.D, E.A. Koetz (2018). Germination biology of button grass (*Dactyloctenium radulans*): An emerging summer grass weed in cotton farming systems. NSW DPI Southern NSW research results, 2018.

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

NA

Part 4 – Final Report Executive Summary

Previous research has highlighted that glyphosate resistant and tolerant weeds are increasing in cotton farming systems. This situation is not unique to cotton systems, indeed broadacre agriculture in Australia is dealing with a range of herbicide resistant weeds across all farming systems. Glyphosate tolerant cotton has been widely adopted in Australia since its introduction in the early 2000’s. A consequence of this technology was a shift to a simplified system of weed control dominated by multiple applications of glyphosate in crop.

A series of comprehensive weed surveys were conducted across cotton farming systems in 2015, 2016 and 2017 seasons. The focus of the surveys was five common weeds identified as the most problematic in cotton farming systems: feathertop Rhodes grass, windmill grass, awnless barnyard grass, sowthistle and fleabane.

The samples were germinated in a glasshouse and screened against the commercial rate of glyphosate. The testing process confirmed very high levels of resistance in fleabane (>95%) windmill grass (>90%) and awnless barnyard grass (>65%). The level of resistance in sowthistle and feathertop Rhodes grass was lower, however in excess of 25% of populations tested as resistant. Group A resistance was also confirmed in grass populations collected during the surveys.

The additional information from the resistance testing was a catalyst for updating the Herbicide Resistance Management Strategy (HRMS). The new strategy includes information on herbicide resistance for: Group M, L, I and A herbicides. A resistance risk assessment tool has also been incorporated into the HRMS table. Feedback from consultants and growers has been positive, especially with the traffic light approach in the table. The new HRMS has been included in the 2018 Cotton Pest Management Guide. In addition, all tables within the Weed Management section have been updated and consolidated into a more user friendly format.

Since the start of the project in 2014 and the extension of herbicide resistance results there has been an increase in the use pattern of pre-emergent and residual herbicides. This is a positive outcome for the industry and further reinforces the need to provide growers with additional options for weed control other than glyphosate. A research officer was appointed to the project in February 2017 further increasing the capacity of weed research in the cotton industry.

A study tour to the US cotton industry in September 2017 reinforced to researchers, growers and industry the importance of maintaining a diverse approach to weed control in Australian cotton farming systems. The reliance on herbicides alone for weed control has resulted in widespread resistance developing in the US, especially to glyphosate. The Australian industry based HRMS (2 + 2 & NO survivors) is essential to maintaining the efficacy of glyphosate for the cotton industry. The importance of non-glyphosate tactics in-crop and in fallow, and controlling any survivors is paramount to the long term sustainability of our cotton farming system.

Phenology studies confirmed that susceptible windmill grass populations accumulated higher levels of shikimate than resistant phenotypes. This work suggests that resistance is likely to be target site based (not confirmed). Low levels of paraquat applied to fleabane populations showed increased growth rates for resistant populations compared to susceptible types (hormesis). One population of fleabane has tested R to paraquat and this is being communicated through future WEEDsmart and Spotlight articles. Dose response experiments on barnyard grass showed that even when exposing plants to high rates of glyphosate they were able to survive by reducing tiller numbers, shortened growth habit and a reduction in seeds per spikelet.

The pupae busting experiment at ACRI has been updated to include a range of integrated weed management tactics to differentiate previous trial treatments. Early indications are of a weed species shift between the control (W3) treatments and the introduction of pre-emergent herbicides.

Eric was appointed as the Weed Management Technical Lead with CottonInfo in February 2017. Eric has also assumed the role as the co-ordinator of the Herbicide Technical Panel. The Technical Lead has responsibility for the Weed component of the Annual Operating Plan for CottonInfo Regional Extension Officers's. A series of weed management case studies was completed in the Summer of 2018 and published on the WeedSmart web site and in industry publications. Eric has participated in the filming of three videos on Herbicide resistance management and integrated weed management.

Industry engagement at workshops, conferences and field days were a key component of the project. A pre-emergent demonstration trial at Whitton NSW highlighted the effectiveness of adding a pre-emergent herbicide especially in the presence of high populations of grass weeds.

The Situation paper "Weeds, Herbicides and Traits in Australian Cotton" is in final draft form and awaiting feedback from reviewers.