Glyphosate resistance in Australian cotton farming systems, what are the surveys telling us? The then and now.

Eric A. Koetz[1], MD. Asaduzzaman[1], Jeff A. Werth[2] and Graham W. Charles[3].
[1] NSW Department of Primary Industries, WWAI, PMB, Pine Gully Road Wagga Wagga NSW, 2650
[2] Leslie Research Centre, Queensland Department of Primary industries, Toowoomba, 4350
[3] NSW Department of Primary industries, ACRI, Narrabri, NSW 2390

Summary
During the last 20 years several industry wide surveys have been conducted to determine the frequency and distribution of common weeds in Australian cotton farming systems. A shift in species occurrence has been recorded with problematic broad leaf weeds replaced with hard to control grass weeds. The three most common in-crop weeds from a 1991 grower survey were, Noogoora burr, Cyperus species and Bathurst burr (Charles, 1991). In 2001 Peachvine, Bladder ketmia and Nutgrass were the top three (Charles, 2001) and in 2010 Flaxleaf fleabane, Sowthistle and Peachvine were the most abundant (Walker *et al.* 2010). In 2015 the three most common weeds recorded were feathertop Rhodes grass, Awnless barnyard grass and Windmill grass.

In addition to the shift in species occurrence, weed surveys conducted since 2015 have confirmed high levels of glyphosate resistance in five hard to control weeds in cotton farming systems. In 2015-16, >95% of Flaxleaf fleabane populations were resistant and in 2017-18, >75% of populations were resistant. For grass weeds Awnless barnyard grass populations from 2015 (>70%), 2017 (65%) and 2018 (>66%). Feathertop Rhodes grass populations are of the most concern with an increase in glyphosate resistance measured from 20% in 2015, 35% in 2017 and 40% in 2018. Resistance levels in Windmill grass were 45% from 2017 and 2018 whilst samples from 2015 were 90% resistant. The high levels of glyphosate resistance are concerning and reinforce the importance of the cotton industry's Herbicide Resistance Management Strategy (HRMS) which promotes a dynamic, integrated approach to weed control focusing on preserving the long-term efficacy of glyphosate.

Keywords glyphosate resistance, species-shift, grass weeds, Herbicide Resistance Management Strategy.

INTRODUCTION

The introduction of Roundup Ready® varieties to Australia in the 2000-01 cotton season was quickly adopted by the industry and now encapsulates 99% of all cotton areas sown. The rapid adoption of this technology around the world coincided with a shift toward minimum and no till farming (Givens, *et al.* 2009). In conjunction with changes to tillage, the inclusion of genetically modified (GM) crops around the world has seen a shift in species composition (Culpepper 2006, and Werth et.al.2013).

Many conventional tactics including in-crop tillage, pre-emergent residual herbicides and hand chipping were replaced with glyphosate as the primary form of weed control. The reliance on glyphosate, often as the only active ingredient used, has resulted in weed species shifts and the evolution of glyphosate resistant weeds in GM crops (Johnson *et al.* 2009). The Australian cotton industry has followed a similar pattern with broadleaf weeds replaced by grass weeds as the most problematic and hard to control.

In the 1990s Australian cotton farmers used a range of chemical and mechanical tools to control weeds in their fields. Weed control relied on large inputs of mostly residual herbicides followed by the inevitable hand chipping to control escapes or survivors. Charles reported in 1991 that the cost of weed control from a survey of growers in 1989 averaged $187 per hectare with herbicides accounting for $76 and chipping $67. In 2018 the Australian Cotton Comparative Analysis reported herbicide cost was $134 per hectare and chipping was $4 per hectare. The relative reduction in costs can be attributed to the introduction of Roundup Ready® Cotton in the early 2000's. This system evolved quickly to a relatively simple weed management plan heavily reliant on glyphosate. However, the impressive control provided by glyphosate may also turn out to be its Achilles heel.

In the United States widespread resistance to glyphosate developed within 10 years of the introduction of glyphosate tolerant varieties. The Australian cotton industry is approaching the 20-year mark since the introduction of Roundup Ready® cotton, and as such a need was identified to quantify the resistance status of key weeds in Australian fields.

Industry surveys have been conducted to try and get an understanding of the level of glyphosate resistance in cotton fields. In a 2016 survey growers reported that they suspected resistance (approximately 74%) to glyphosate, however resistance testing is only at relatively low levels (30%). A follow up survey in 2019 has seen an increase in suspected resistance, with 85% of growers reporting issues with weed control. This is supported with 52% of growers confirming glyphosate resistant weeds in the 2020-21 season (CCA Qualitative report, 2020-21).

To validate these grower results funding from the Cotton Research and Development Corporation and NSW Department of Primary Industries allowed researchers to collect samples from cotton fields for herbicide resistance testing to quantify these findings.

MATERIALS AND METHODS

In the 2015-16 cotton season a random survey was conducted sampling 144 fields on 50 farms across 7 cotton farming regions in Queensland and NSW. Sampling was coordinated to occur after post-emergent application of glyphosate to collect seed from survivors and a record of other weeds present at each site was noted. Targeted weed surveys from 43 fields were conducted in 2017 and 70 samples were collected across cotton growing regions in 2018/19. In addition to the collection of weed seeds a record of the incidence of weed species was also compiled for each field.

Seeds of these populations were sown on soil surface of plastic pots (25 cm in diameter) pre-filled with potting mix with the top 2 cm field soil. Seeds were placed on the substrate surface, watered, and covered with paper towel and maintained in a glasshouse. Plants from each pot were transplanted to trays with same peat substrate (6 alternating spots on the tray) at two to four-leaf stage. Each population had 18 experimental units (6 plants or units per replication).When seedlings were at the rosette stage (8-10 cm diameter for broadleaf weeds and early tillering for grass weeds), they were sprayed with 0.68 kg ae ha^{-1} of glyphosate,http://www.wssajournals.org/doi/full/10.1614/WT-D-09-00080.1 - n3#n3 which is a commonly used rate for general fallow weed control in Australia (Walker *et al*. 2011).

The herbicides were 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. The irrigation was turned off one day prior to spraying and turned back on one day post spray application. Trays were arranged in a completely randomized design with three replications. Seedling survival was assessed at 28 days after treatment (DAT) using a scale ranging from 0% (zero control or no difference from control) to 100% (complete control or plant death). Total number of survived plants for each population was counted and converted to a percentage value at 28 DAT. Populations with plant survival >20% after spraying was considered ‘‘resistant’. Plant survival of 10% to 19% was ‘‘developing resistance” and populations with plant death and necrosis >90% or survived less than 10% considered as “susceptible”.

RESULTS

Results from the 2015 survey for sowthistle recorded over 20% assessed as resistant or developing resistance to glyphosate and remaining at similar levels across all three surveys (2017/18 survey only had 6 viable samples tested). Very high levels of glyphosate resistance (>95%) were recorded in Fleabane in 2015 and greater than 75% in 2016 and it appears to have a naturally high tolerance to glyphosate, and as such has not been included in further surveys. Windmill grass and Barnyard grass are either developing or have high levels of resistance to glyphosate (Table 1).

The biggest concern from the surveys has been the increase in glyphosate resistance in feathertop Rhodes grass samples collected with an increase in three seasons from 20% to 40% of samples resistant (Table 1).

Table 1. Percentage of populations of six problem weeds resistant to glyphosate from surveys in 2015 to 2018.

Weeds	2015/16	2016/17	2017/18
Fleabane	97	75	Not tested
Sowthistle	22	10	28
Barnyard grass	72	65	57
Windmill grass	90	45	44
Feathertop Rhodes grass	20	35	40
Annual ryegrass	Not tested	Not tested	83

From the historical to the most recent surveys there has been a significant shift in species prominence from the broadleaf weeds of the pre-Roundup Ready® era to an increase in grass weeds (Table 2). The increase in glyphosate resistance in grass weeds follows a similar pattern to that observed in minimum till broadacre grain systems in northern NSW. In the latest surveys we are now starting to see fleabane and sowthistle hard to control, especially in dryland cotton systems where minimum or zero tillage dominates.

Table 2. The three most common weed species through time in Australian cotton fields

1991	2001	2010	2016
Noogoora burr	Peachvine	Flaxleaf fleabane	feathertop Rhodes grass
Cyperus spp	Bladder ketmia	Sowthistle	Awnless barnyard gass
Bathurst burr	Nutgrass	Peachvine	Windmill grass

DISCUSSION

The surveys have identified weeds that are developing glyphosate resistance. The reliance on glyphosate as the main weed control tactic has placed increased selection pressure from using a single mode of action. The result is a species shift from fields with mostly broadleaf weeds (Werth et.al.2013) to the recent surveys showing an increase in grass weeds. Over 80% of growers are using glyphosate as the only knock down herbicide prior to planting, this coupled with in-crop applications, places a lot of pressure on glyphosate to do all the heavy lifting for weed control. Many weeds are now proving difficult to control in a glyphosate dominant system.

Consequently, we have seen a shift in species occurrence with grass weeds the most common survivors of glyphosate applications. In response we are seeing a move to more diverse weed control tactics including pre-emergent, in-crop and layby herbicides with different modes of action. The addition of targeted tillage, shrouded sprayers and optical spray technology has added extra diversity and robustness to weed control.

Results from the latest CCA surveys report growers are using a diverse range of herbicides across a number of MOA's The CCA qualitative report from 2020-21 reports that almost 70% of growers are applying a pre plant residual and 43% are applying a residual herbicide at planting. Additionally, 64% of growers are incorporating more than two and three other modes of action into their weed control program, however what is concerning is that we still see 33% of growers using glyphosate plus only one other mode of action.

The Australian cotton industry has been very proactive in developing a stewardship program around integrated weed management. It is just as important to target the non-cropping phase of the rotation and implement robust and diverse tactics for weed control, including the use of at least two non-glyphosate herbicides in fallow and in crop (Thornby *et el.* 2013). The addition of in-crop tillage has also proven to be a useful tool for controlling late emerging weeds in crop and controlling survivors of herbicide application. This approach is the cornerstone of the (HRMS) developed by the cotton industry.

As a result of the stewardship adopted by Australian cotton growers, we still have good efficacy with glyphosate. We have detected a species shift toward grass dominant weed communities which have proven difficult to control with glyphosate alone. Of greatest concern is the increase in glyphosate resistance for feathertop Rhodes grass. This hard to control weed is spreading across the whole farming system and is now the weed of greatest concern amongst growers.

The weed survey results are important to guide decision making now and into the future as we grapple with developing glyphosate resistance.

The importance of an integrated approach to weed management. is critical to protect the long - term efficacy of glyphosate. As always, the focus remains on controlling all survivors from glyphosate applications

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