Species shift and resistance: challenges for Australian cotton systems

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Summary Weed control practices in the Australian cotton industry, both in crop and fallow, are now heavily reliant on glyphosate. Even before the introduction of glyphosate-tolerant varieties, glyphosate was increasingly being used for pre-plant knockdowns, and shielded sprays within the crop. To determine any changes in species composition resulting from this reliance on glyphosate, we re-surveyed 50 fields previously surveyed in 2001. Results showed a species shift, with flaxleaf fleabane and sowthistle now dominating the flora in cotton systems. Other glyphosate-tolerant species, such as Australian bindweed, have also increased in a number of fields. Bladder ketmia, peachvine and awnless barnyard grass were also common in the majority of fields. A further threat to the industry was the identification of the first glyphosate-resistant weed in Australian cotton systems, awnless barnyard grass. This paper outlines these challenges for the industry, and explores a range of chemical management options for these glyphosate-tolerant and resistant weeds.

Keywords Glyphosate resistance, species shift, survey.

INTRODUCTION

Weed management practices in the Australian cotton industry have changed from systems based around tillage, to minimum or zero-tillage systems based on glyphosate and permanent beds in irrigated systems (Charles *et al*. 2004). Even before the introduction of glyphosate-tolerant varieties, glyphosate was becoming commonly used for pre-plant knockdown applications, and shielded applications within the crop. Glyphosate use in fallow has largely replaced tillage, particularly in dryland systems.

Previous surveys conducted in cotton-based farming systems (Charles *et al*. 2004, Walker *et al*. 2005) had already shown some shift towards glyphosate-tolerant, small seeded species favoured by frequent glyphosate use and little or no tillage. The introduction of glyphosate-tolerant cotton in 2000 has created an even greater reliance on glyphosate.

In this study, fields that had previously been surveyed by Charles *et al*. (2004) and Walker *et al*. (2005) were revisited in order to identify any further changes to the weed spectrum since the introduction of glyphosate-tolerant cotton. The threat of glyphosate resistance to the industry became a reality in 2009 with a resistant awnless barnyard grass (*Echinochloa colona* (L.) Link) population being identified in a cotton rotation. Experiments to further understand this population and to determine the effectiveness of glyphosate alternatives in cotton systems were undertaken.

MATERIALS AND METHODS

Field surveys Fifty fields were selected that had been previously surveyed, 26 from Walker *et al*. (2005) and 24 from Charles *et al*. (2004). The fields were located in the Darling Downs and McIntyre valleys in Queensland, and the Gwydir and Lower Namoi valleys in New South Wales.

Two surveys were done, the first at the start of the summer cropping season (November–December 2008) and the second at the end of the same season (March–April 2009). Surveys were done at these times to gain an understanding of what weed species were present at the time when the majority of herbicides are applied, and to identify weeds that either germinated later in the season or were not controlled.

Surveys were done in a similar manner to Walker *et al*. (2005). Fields were divided into three to four sections depending on the size and shape of the fields. Transects with quadrats approximately 50 m apart were surveyed in each section so that a total of 20 quadrats per field were surveyed. Quadrats were 10 × 1 m. The presence and density of each weed species were noted in each quadrat with species density rated using the scale 0–3: 0 = no weeds 10 m^{-2}; 1 = 1–9 weeds 10 m^{-2}; 2 = 10–100 weeds 10 m^{-2}; 3 = >100 weeds 10 m^{-2}.

Barnyard grass studies Seed from the suspected resistant site, on the Darling Downs in Queensland, was collected from surviving plants after a glyphosate application at what was considered to be a robust rate of 820 g a.e. (acid equivalent) glyphosate ha^{-1} (plants were sprayed at mid-tillering). At the time of collection, the field was in the fallow phase of a non-irrigated cotton-fallow rotation. Care was taken to avoid wheel tracks and other areas where control levels could be confused with other factors. A dose response experiment in pots was undertaken to compare this population (QBG4) with a known susceptible population (QSBG1) (5 pots × 3 plants per pot). Glyphosate rates were 0, 90, 135, 180, 225, 315 and 450 g a.e. glyphosate ha^{-1} with spray volume of 75 L ha^{-1} of water. Plants were sprayed at two tillers. Survivors were counted 28 days after application. This experiment was repeated.

A dose response experiment was also undertaken at the field site. Glyphosate rates were 0, 45, 90, 180, 360, 720 and 1440 g a.e. glyphosate ha^{-1} with a spray volume of 75 L ha^{-1}. Two sizes were present and measured at the time of spraying, approximately four leaf and three tillers. Survival counts were taken 21 days after application

RESULTS AND DISCUSSION

Field surveys At the time of the survey 31 fields were in fallow, 12 in sorghum, four in cotton, two in maize and one in sunflowers. Over 70 species were observed in the surveys with the majority of them being present in low densities.

The greatest change since the 2001 surveys was the increase in flaxleaf fleabane (*Conyza bonariensis* (L.) Cronq.). In the surveys conducted by Walker *et al*. (2005) (Table 1) flaxleaf fleabane was ranked at 14th compared to 2nd in 2009. Flaxleaf fleabane did not rank in the top 20 in surveys conducted by Charles *et al*. (2004) and in 2009 was ranked 2nd in the same fields (Table 2). We suspect this is due to minimum tillage cropping systems with a heavy reliance on glyphosate.

Flaxleaf fleabane is poorly controlled by glyphosate and only emerges from the top 0.5 cm of soil. Further changes in the weed spectrum, noted by Charles *et al*. (2004) with the adoption of permanent beds, a reduction in cultivation and a reliance on glyphosate, were observed in the current survey. The weed species observed in 2001 are similar to those observed in 2009 (apart from fleabane) indicating the similar farming practices being used.

In the fields surveyed by Charles *et al.* (2004), common sowthistle (*Sonchus oleraceus* L.) was ranked 13th and increased to 3rd in 2009. It was also ranked high in the Walker *et al*. (2005) survey. Like flaxleaf fleabane, common sowthistle is a small seeded Asteraceae that is favoured by no-till cropping systems (Charles *et al*. 2004). It has the ability to germinate year round, making it a difficult weed to get season long control.

Bladder ketmia (*Hibiscus trionum* L.) continues to be one of the major weeds in all fields and years. Bladder ketmia seed is persistent with previous studies showing that after 36 months of burial at 10 cm over half of the seeds were still viable (Walker *et al*. 2010). It can also emerge from the surface down to 5 cm deep. Therefore it stays in the seed bank for long periods.

Barnyard grass studies The pot dose response experiment showed greater survival after spraying with glyphosate of the QBG4 population than the QSBG4 population (Figure 1). Our results and further testing by the University of Adelaide (Chris Preston pers. comm.) showed a low-level resistance in the order of 2.5–3 fold. The LD_{50} for QSBG1 and QBG4 was approximately 105 and 315 g a.e. glyphosate ha^{-1} respectively.

This level of resistance is similar to what has been found in other resistant awnless barnyard populations in northern New South Wales (Chris Preston pers. comm.) indicating a similar mechanism, possibly a weak target-site modification.

The field testing of the QBG4 population only showed a difference between the two plant sizes of four leaf and three tillers at lower rates (Figure 2). At the highest rate of 1440 g glyphosate ha^{-1} both sizes had approximately 1.5% survival. This rate is equivalent to 3.2 L ha^{-1} of Roundup CT® and 1.44 kg ha^{-1} of Roundup Ready® Herbicide (690 g a.e. glyphosate kg^{-1} formulation) which is registered for use in glyphosate-tolerant (Roundup Ready Flex®) cotton.

Due to the low-level resistance of this population, higher rates of glyphosate will still provide control to small plants under ideal conditions. However, if plant size increases and/or conditions aren't favourable, the highest rate of Roundup Ready herbicide allowed in Roundup Ready Flex cotton (1.5 kg ha^{-1}) will struggle to provide effective control. Therefore applications withheld until later in the season in order to control multiple cohorts with the one application may increase the risk of poor control on resistant plants in crop, particularly if there are larger plants present. In addition, earlier applications at higher rates may mask any potential resistance evolution (this could have been a contributing factor in the current situation). It is therefore critical the effectiveness of applications be monitored and survivors controlled to ensure seed production is stopped.

In fallow situations, glyphosate is still the herbicide of choice. The double knock tactic (sequential

Table 1. Comparison of top 15 weeds present in fields surveyed by Walker *et al.* (2005) to the 2009 surveys, along with the presence and mean density rating of weeds at the start and end of the 2009 season.

2001		2009		Start of 2009 season		End of 2009 season	
Rank	Weed species	Rank	Weed species	% fields present	Mean density	% fields present	Mean density
1	*Hibiscus trionum*	1	*Hibiscus trionum*	77.3	0.5	36.0	0.3
2	*Sonchus oleraceus*+	2	*Conyza bonariensis*[A,B]	59.1	0.4	48.0	0.3
3	*Portulaca oleracea*	3	*Sonchus oleraceus*[B]	59.1	0.5	64.0	0.4
4	*Tribulus* spp.	4	*Tribulus terrestris*	27.3	0.1	12.0	0.1
5	*Amaranthus macrocarpus*[A]	5	*Echinochloa colona*	22.7	0.4	28.0	0.8
6	*Echinochloa* spp.	6	*Ipomea lonchophylla*[A,B]	22.7	0.2	20.0	0.2
7	*Ipomea lonchophylla*[A,B]	7	*Portulaca oleracea*	22.7	0.3	0.0	0.0
8	*Convolvulus erubescens*[A,B]	8	*Amaranthus macrocarpus*[A]	18.2	0.1	12.0	0.1
9	*Chamaesyce drummondii*	9	*Convolvulus erubescens*[A,B]	18.2	0.1	24.0	0.4
10	*Urochloa panicoides*	10	*Urochloa panicoides*	18.2	0.1	4.0	0.4
11	*Amaranthus mitchelli*	11	*Brachiaria* sp.	13.6	0.1	0	0.0
12	*Malvastrum americanum*[A]	12	*Chamaesyce drummondii*	13.6	0.1	20.0	0.1
13	*Cucumis anguria*[A]	13	*Chloris virgata*[A]	13.6	0.3	28.0	0.2
14	*Conyza bonariensis*[A,B]	14	*Echinochloa crus-galli*	13.6	0.2	0	0.0
15	*Physalis minima*	15	*Lactuca serriola*	13.6	0.1	0.0	0.0

[A] Not on Roundup Ready® herbicide label.
[B] Weeds that have a naturally high level of tolerance to glyphosate (Charles *et al.* 2004).

Table 2. Comparison top 15 weeds present in fields surveyed by Charles *et al.* (2004) to the 2009 surveys, along with the presence and mean density rating of weeds at the start and end of the 2009 season.

2001		2009		Start of 2009 season		End of 2009 season	
Rank	Weed species	Rank	Weed species	% fields present	Mean density	% fields present	Mean density
1	*Ipomea lonchophylla*[A,B]	1	*Hibiscus trionum*	65.2	0.3	9.5	0.0
2	*Hibiscus trionum*	2	*Conyza bonariensis*[A,B]	47.8	0.3	23.8	0.3
3	*Cyperus rotundus*[B]	3	*Sonchus oleraceus*[B]	47.8	0.2	0.0	0.1
4	Echinochloa colona	4	*Convolvulus erubescens*[A,B]	43.5	0.4	28.6	0.0
5	*Rhyncosia minima*[A,B]	5	*Ipomea lonchophylla*[A,B]	39.1	0.3	42.8	0.0
6	*Cullen cinereum*[A,B]	6	*Cullen cinereum*[A,B]	30.4	0.4	9.5	0.5
7	*Gossypium hirsutum*[A]	7	*Tribulus terrestris*	26.1	0.2	0.0	0.2
8	*Physalis* sp.	8	*Cyperus* sp.[B]	17.4	0.3	0.0	0.1
9	*Datura ferox*	9	*Echinochloa colona*	17.4	0.2	14.3	0.1
10	*Neptunia gracilis*[A,B]	10	*Fallopia convolvulus*	17.4	0.8	0.0	0.0
11	*Convolvulus erubescens*[A,B]	11	*Lactuca serriola*	17.4	0.1	4.8	0.0
12	*Polymeria pusilla*[A]	12	*Rhyncosia minima*[A,B]	17.4	0.1	0.0	0.2
13	*Sonchus oleraceus*[B]	13	*Vigna lanceolata*[A,B]	17.4	0.2	9.5	0.0
14	*Sesbania cannibina*[A]	14	*Amaranthus macrocarpus*[A]	13.0	0.2	23.8	0.0
15	*Xanthium* sp.	15	*Avena* spp.	13.0	0.1	0.0	0.0

[A] Not on Roundup Ready herbicide label.
[B] Weeds that have a naturally high level of tolerance to glyphosate (Charles *et al.* 2004).

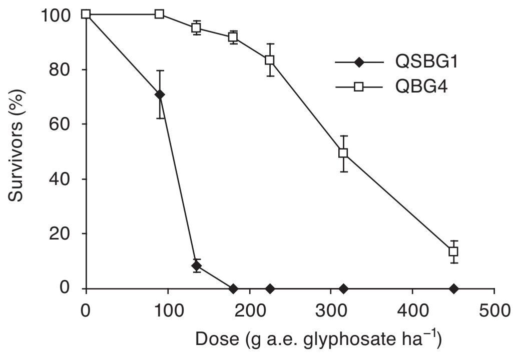

Figure 1. Dose response of 'resistant' (QBG4) and 'susceptible' (QSBG1) barnyard populations to glyphosate.

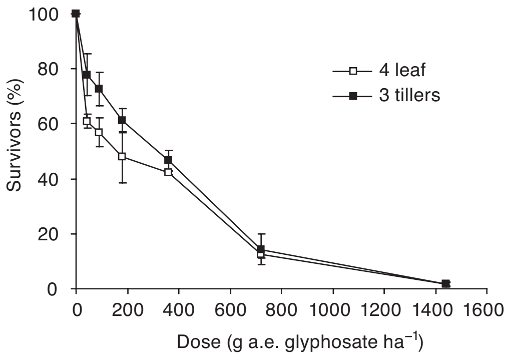

Figure 2. Dose response of the 'resistant' QBG4 barnyard grass population to glyphosate in the field.

knockdown herbicide applications from different herbicide mode-of-action groups, commonly glyphosate followed by paraquat) will provide a method of controlling survivors and reducing seed set in these situations (Werth *et al*. 2008) and has become an important tool for managing fleabane as well. The other options for fallow control are very limited. Group A herbicides have a high resistance risk, and therefore should be left for within crop applications. Tillage is often only considered an option in salvage situations.

The Australian cotton industry faces real challenges with the ever growing problem of fleabane and this new case of resistant awnless barnyard grass. The value of glyphosate-tolerant technology to the cotton industry has not diminished, as glyphosate is still an important herbicide for managing a broad spectrum of weeds within crop that were much harder to manage before its introduction. However, in light of the present paper and similar findings, it is important that alternatives to glyphosate are still incorporated as part of an integrated weed management (IWM) program. Herbicides such as pre-emergence residuals (including metolachlor, pendimethalin and diuron) and post-emergent herbicides from different mode of action groups are still vitally important in a glyphosate-tolerant cotton system. Tillage in the form of inter-row cultivation, bed preparation and management of irrigation furrows needs to continue to be included in an IWM program to ensure the sustainability of glyphosate in cotton systems.

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