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Assessing weeds at risk of evolving glyphosate resistance in Australian sub-tropical glyphosate-resistant cotton systems

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Abstract. Glyphosate resistance will have a major impact on current cropping practices in glyphosate-resistant cotton systems. A framework for a risk assessment for weed species and management practices used in cropping systems with glyphosate-resistant cotton will aid decision making for resistance management. We developed this framework and then assessed the biological characteristics of 65 species and management practices from 50 cotton growers. This enabled us to predict the species most likely to evolve resistance, and the situations in which resistance is most likely to occur. Species with the highest resistance risk were *Brachiaria eruciformis*, *Conyza bonariensis*, *Urochloa panicoides*, *Chloris virgata*, *Sonchus oleraceus* and *Echinochloa colona*. The summer fallow and non-irrigated glyphosate-resistant cotton were the highest risk phases in the cropping system. When weed species and management practices were combined, *C. bonariensis* in summer fallow and other winter crops were at very high risk. *S. oleraceus* had very high risk in summer and winter fallow, as did *C. virgata* and *E. colona* in summer fallow. This study enables growers to identify potential resistance risks in the species present and management practices used on their farm, which will to facilitate a more targeted weed management approach to prevent development of glyphosate resistance.

Additional keywords: glyphosate resistance, glyphosate-resistant cotton, risk assessment.

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Introduction

Glyphosate-resistant (GR) cotton was introduced in the Australian cotton industry in the 2000–01 season. Since then it has been widely adopted with 95% of cotton planted being GR in 2009–10. In-crop applications of glyphosate allowed improved control of some difficult-to-control weeds. The ability to substitute glyphosate for some conventional inputs, such as pre-emergence residual herbicides helped to reduce the risk of early-season damage and poor growth of cotton seedlings due to heavy rainfall concentrating the residuals in the seed zone (Charles *et al*. 1995). Both these factors have contributed to its rapid adoption. Even before the introduction of GR 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 non-irrigated systems.

Glyphosate resistance has evolved in five weed species in the sub-tropical cropping region of north-east Australia – *Lolium rigidum* Gaud., *Echinochloa colona* (L.) Link, *Urochloa panicoides* P. Beauv., *Conyza bonariensis* (L.) Cronquist and *Chloris truncata* R.Br. (Heap 2011; Preston 2011). Effective management of these species to ensure their control is vitally important. However, as many cropping fields contain a diverse range of weed species, other species may also be at risk to evolving glyphosate resistance. Over 100 weed species have been identified in fields in this grain and cotton cropping region (Charles *et al*. 2004; Rew *et al*. 2005; Walker *et al*. 2005).

Clearly, however, not all weed species exposed to selection with glyphosate have so far developed glyphosate resistance. Several factors influence the evolution of herbicide resistance in weed populations. These are derived from genetic and biological characteristics of the weed species, and the management practices applied to the population (Powles and Yu 2010).

The species characteristics in particular that contribute to the evolution of herbicide resistance are gene mutation rate, initial frequency of resistance genes, inheritance, mating systems and gene flow (Jasieniuk *et al*. 1996). Although mutation rates and initial frequencies differ between herbicide modes of action (Maxwell and Mortimer 1994; Preston and Powles 2002; Neve *et al*. 2003), these frequencies are assumed to be similar between species, particularly in the construction of resistance models (Jasieniuk *et al*. 1996; Werth *et al*. 2008; Thornby and Walker 2009).

Herbicide resistance will spread more rapidly in cross-pollinated populations when associated with a single dominant allele (Maxwell and Mortimer 1994). However, selfing in a plant can increase the probability of the rate of evolution of resistance conferred by recessive alleles (Jasieniuk *et al*. 1996). The influence of mating behaviour (cross-pollinated or self-pollinated) on the evolution of resistance will depend on the nature of inheritance of resistance alleles (Jasieniuk *et al*. 1996).

Generation turnover is also a highly important factor in the rate of resistance evolution (Stanton *et al*. 2008). *Eleusine indica* (L.) Gaertner is a prolific seed producer and has four generations per year in Malaysia (Powles and Preston 2006), as a result glyphosate resistance evolved under persistent glyphosate usage in 3 years (Lee and Ngim 2000). This compares with *L. rigidum* in Australia, which evolved resistance after 15 years with one generation per year (Powles *et al*. 1998). The number of generations in each case was similar, 12 and 15, respectively. Although glyphosate was applied substantially more times per year on *E. indica* than *L. rigidum*, the frequent generation turnover also played a major role in the rate of resistance evolution.

Dense weed populations have a higher probability of developing resistance, even when the rate of mutation is low (Jasieniuk *et al*. 1996; Diggle *et al*. 2003). Species with high fecundity (either vegetatively, by seed or both) that have minimal seed dormancy tend to result in dense weed populations (Benech-Arnold *et al*. 2000).

Seed production of *L. rigidum*, *E. colona*, *U. panicoides*, and *C. bonariensis* can reach totals of over 30 000, 12 000, 2000 and 100 000 seeds/plant, respectively (Mercado and Talata 1977; Pannell *et al*. 2004; Wu *et al*. 2007; Werth *et al*. 2008). *C. bonariensis* has virtually no dormancy (Green *et al*. 2008), and although *E. colona*, *U. panicoides*, and *L. rigidum* have moderate levels of dormancy (Steadman *et al*. 2003; Kovach *et al*. 2010), they germinate at relatively specific times of the year, which often results in dense populations (Pannell *et al*. 2004; Werth 2007; Thornby and Walker 2009). This combined with persistent glyphosate use has resulted in them being selected for glyphosate resistance.

The other major determinant for resistance evolution is selection pressure. Species characteristics combined with continuous use of one or a few herbicides contributes to selection of resistance alleles (Maxwell and Mortimer 1994; Jasieniuk *et al*. 1996). Herbicide resistance appears where one or a few herbicides were used persistently to manage weeds (Preston and Rieger 2000; Stanton *et al*. 2008). Modelling has shown that using other herbicides as substituted for and in addition to glyphosate can delay resistance evolution (Diggle *et al*. 2003; Werth *et al*. 2008). Selection pressure for glyphosate resistance is a function of the frequency of glyphosate application and the frequency and effectiveness of other chemical and non-chemical kill methods such as tillage and crop competition (Thornby *et al*. 2010).

It is impractical for growers to monitor all weed species present in their fields. This has resulted in growers targeting a smaller number of key species and assuming that acceptable control will be achieved on the others. Determining which species are important often depends on several factors such as competitiveness with the crop, contamination of grain and lint, noxious weeds and aesthetics. Glyphosate resistance will increase the cost of weed control in addition to these factors. If we can identify species that are more prone to resistance evolution than others, growers can then concentrate their monitoring on those species to reduce the risk of glyphosate resistance evolving in their fields.

In this paper we describe the construction of a risk assessment framework for weeds likely to develop glyphosate resistance in farming systems with GR cotton. This assessment combined biological characteristics of 65 weed species with management practices used by 50 surveyed growers. The identified potential resistance risks derived from the species present in combination with the management practices used on their farm will enable growers to facilitate a more targeted and preventive weed management approach.

Materials and methods

The assessment framework consisted of two components: the biological characteristics of weed species and the management/control practices applied to those species in farming systems with GR cotton grown in southern Queensland and northern New South Wales, Australia.

The risk assessment framework uses an expert systems approach, which is a method commonly used in basic decision support systems and management tools in a variety of fields. The approach has been used in many fields to provide tools for risk assessment and management, including pest (Potter *et al*. 2000) and weed management (Stigliani and Resina 1993; Monks *et al*. 1995; Wilkerson *et al*. 2002). Expert systems typically use a questioning or survey approach to gather information about an individual case, such as herbicide usage on weeds at a paddock level, from a non-expert user, and interpret the results using a series of statements or steps designed to reproduce the way a human expert would analyse them. The assessments used in expert systems may be based on interpreted data where available or, similarly to Bayesian belief networks, the consensus opinion of experts (Spiegelhalter *et al*. 1993). In our case we analysed a combination of real data, assumptions from existing resistance cases, and modelling (Werth *et al*. 2008; Thornby and Walker 2009) to produce a weighted scoring system. The framework uses our expertise-derived weightings to assess resistance risk for each weed or management scenario.

Species risk

Sixty-five species were included in the risk assessment as they were either named on glyphosate labels in Australia, known to have glyphosate resistance internationally, or found in various field surveys conducted in the northern grain region of Australia (Charles *et al*. 2004; Walker *et al*. 2005).

Species resistance potential was assessed using five main characteristics (Jasieniuk *et al*. 1996; Powles and Yu 2010). These were fecundity (F_i), proportion of the viable seed bank that generally emerges (P_i), ability to outcross (M_i), method of reproduction (S_i), and generation period (T_i). The importance of these biological characteristics was determined by weighting categories using the expert systems approach (Table 1). For the purposes of this assessment it was assumed that resistance is nuclear, dominant and conferred by a single gene. This

Table 1. Weed species characteristics and corresponding weightings used for the species risk assessment for evolution of glyphosate resistance

Characteristic	Category	Weighting
1. Fecundity	>100 000 seeds/plant	10
	10 000–100 000 seeds/plant	6
	1000–10 000 seeds/plant	3
	<1000 seeds/plant	1
2. Proportion of viable seed bank emerging	Large with a single cohort	10
	Large with multiple cohorts	9
	Medium with a single cohort	6
	Medium with multiple cohorts	5
	Small	0
3. Mating	Mostly selfing	0.5
	Both selfing and outcrossing	1
	Mostly outcrossing	1
4. Reproduction method	Sexual	1
	Vegetative	0.5
	Both	1
5. Generation period	Annual species with multiple generations per year on most seed produced	5
	Annual species with multiple generations per year on some seed produced	2
	Annual with one generation per year	0
	Perennial	0

assumption was based on the fact that, in previous cases studied, a single gene confers glyphosate resistance (Lorraine-Colwill *et al.* 2001; Zelaya *et al.* 2004).

For each species (i), categories were weighted, and added to form a score R_{S_i}, which is calculated as:

$$R_{S_i} = F_i + P_i + M_i + S_i + T_i \tag{1}$$

The scoring system for *E. colona* is demonstrated in Table 2. The theoretical maximum score achievable was 27, the species results were indexed against this maximum to produce a score out of 10 (as demonstrated in Table 2).

Management risk

The management risk associated with different parts of the crop rotation, R_{M_j} (where j is the phase or unit of the rotation i.e. summer fallow, or cotton crop etc.) is calculated as:

$$R_{M_j} = G_j - (C_j * E_{C_j}) - (A_j * E_{A_j}) - K_J \tag{2}$$

where G_j is the number of glyphosate applications per phase j, C_j is the number of times the grower attempts to control glyphosate survivors in phase j, E_{C_j} is the average effectiveness of methods used to control survivors in phase j; A_j is the number of non-glyphosate kill methods used in phase j and E_{A_j} is for the average effectiveness of those alternatives. The values for E_{C_j} and E_{A_j} range from zero to 0.95, this value was chosen as a maximum as it was considered quite difficult to achieve 100% control, particularly on a large scale.

K_j is a crop rating, allowing for a reduction in weed seed set due to competitiveness of the crop use in phase j, K_j is determined as:

$$K_j = K_{F_j} * K_{R_j} * K_{D_j} \tag{3}$$

where K_F is a factor for the type of crop. For the range of rotational phases we studied, K_F ranged from zero for fallow, (which has no competitive effect) to 0.6 for barley, (which has a high competitive effect) (Keeley and Thullen 1991; Lemerle *et al.* 1995; McGillon and Storrie 2006; Werth 2007; Wu *et al.* 2010). K_{R_j} is a reducing factor for crop row spacing, and K_{D_j} is a reducing factor for crop density. Total values for K_j range between zero and 0.6 (Table 3).

Overall risk

The overall risk is determined by the species risk $R_{S_{ij}}$ and the level of selection pressure (management risk) exerted by the cropping system $R_{M_{ij}}$. Species were matched to relevant crops i.e. summer and winter except where growers indicated otherwise. A total risk

Table 2. Risk assessment scoring for *Echinochloa colona* using criteria in Table 1

Characteristic	Category	Weighting
1. Fecundity	10 000–100 000 seeds/plant	6
2. Proportion of viable seed bank emerging	Large with multiple cohorts	9
3. Mating	Mostly selfing	0.5
4. Reproduction method	Sexual	1
5. Generation period	Annual species with multiple generations per year on some seed produced	2
Total		18.5
Indexed out of 10	(18.5/27)*10	6.9

Table 3. Crop competition scoring factors used to determine impact of crop competition on management risk scores

Crop	Competition factor	Row configuration[A]	Row configuration factor	Crop density[B]	Density factor
Irrigated cotton	0.2	Solid	1	High	1
Dryland cotton	0.1	Single skip	0.9	Medium	0.9
Sorghum	0.2	Double skip	0.8	Low	0.8
Sunflowers	0.1	Super singles	0.7	–	–
Maize	0.2	–	–	–	–
Wheat	0.4	–	–	–	–
Barley	0.6	–	–	–	–
Oats	0.4	–	–	–	–
Fallow	0	–	–	–	–
Other summer	0.2	–	–	–	–
Other winter	0.1	–	–	–	–

[A] Row configuration for cotton, sorghum, maize and sunflowers is as follows: solid = 1-m row spacing; single skip = 2 rows planted (1 m apart), 1 row missing; double skip = 2 rows planted, 2 rows missing; super singles = 1 row planted, 3 rows missing.

[B] Crop density relative to accepted industry average.

score $R_{T_{ij}}$ for weed species *i* can then be calculated by multiplying the weed's species score by the average for phase *j*.

$$R_{T_{ij}} = R_{S_i} * R_{M_j} \tag{4}$$

Grower information

In a case study, 50 growers were surveyed to gain information of species present and management practices. Information gained was:

1. Weed species present in crop and fallow;
2. Crops grown, row configuration and planting density;
3. Number of glyphosate applications in crop and fallow;
4. Number of alternatives to glyphosate used in crop and fallow; and
5. Number of times survivors of glyphosate application were controlled, and the effectiveness of those applications.

This information was used to form the management risk component of the assessment.

Results

Species risk

The indexed species risk scores ranged from 8.2 for *Bracharia eruciformis* (Sm.) Griseb. to 0.2 for *Cyperus* spp. The 20 highest scoring species are listed in Table 4. Four of the top 10 species currently have glyphosate-resistant populations in the sub-tropical cropping region of north-east Australia (*C. bonariensis*, 7.6; *U. panicoides*, 7.2; *E. colona*, 6.9; *L. rigidum* 6.3). The other glyphosate-resistant species in this region, *C. truncata*, scored 5.9. *Eleusine indica* (L.) Gaertn. and *Sorghum halepense* L. Pers., which have glyphosate-resistant populations worldwide, were ranked equal 7th and 17th with scores of 6.3 and 3.7, respectively. The lower 45 species not listed in Table 4 had scores less than 3, and as a result are considered to have a low risk of evolving glyphosate resistance.

Other species in the top 20 that have evolved resistance to other herbicide groups include *Avena* spp. (ACCase inhibitors, ALS inhibitors), *Hordeum* spp. (ACCase inhibitors, Bipyridiliums),

Table 4. Species risk scores for the top 20 species identified as being at risk of evolving glyphosate resistance

Species	Common name	Score	References for biological characteristics
Brachiaria eruciformis	Sweet summer grass	8.2	–
Conyza bonariensis	Flaxleaf fleabane	7.6	Wu *et al.* (2007)
Urochloa panicoides	Liverseed grass	7.2	Werth *et al.* (2008)
Chloris virgata	Feathertop Rhodes grass	7.0	Osten (2008)
Sonchus oleraceus	Sowthistle	6.9	Hutchinson *et al.* (1984); Widderick *et al.* (2010)
Echinochloa colona	Awnless barnyard grass	6.9	Mercado and Talata (1977)
Eleusine indica	Crowsfoot grass	6.3	Chin and Raja Harun (1980)
Phalaris paradoxa	Paradoxa grass	6.3	Walker *et al.* (2001)
Hordeum spp.	Barley grass	6.3	–
Lolium rigidum	Annual ryegrass	6.3	Pannell *et al.* (2004)
Dactyloctenium radulans	Button grass	5.9	–
Digitaria ciliaris	Summer grass	5.9	Kobayashi and Oyanagi (2005)
Chloris truncata	Windmill grass	5.9	–
Amaranthus hybridus	Redshank	4.8	–
Cirsium vulgare	Spear thistle	4.8	Sindel (1991); Suwa *et al.* (2010)
Silybum marianum	Variegated thistle	4.8	Sindel (1991)
Sorghum halepense	Johnson grass	3.7	Scopel *et al.* (1988); Vila-Aiub *et al.* (2007)
Eragrostis cilianensis	Stink grass	3.7	–
Avena spp.	Wild oats	3.5	Walker *et al.* (2001)
Lactuca serriola	Prickly lettuce	3.5	–

Phalaris paradoxa L. (ACCase inhibitors), *Sonchus oleraceus* L. (ALS inhibitors), and *Lactuca serriola* L. (ALS inhibitors, Phenoxys) (Heap 2011). Species with herbicide-resistant populations that ranked lower than the highest 20 were *Fallopia convolvulus* (L.) A. Love, *Raphanus raphanistrum* L. and *Sisymbrium thellungii* O. Schultz all with scores of 1.9.

Management risk

Management risk scores averaged across all respondents ranged from 1.5 for non-irrigated GR cotton and summer fallow to 0.2 for irrigated non-GR cotton (Table 5), with the highest individual scores of 5 recorded for summer and winter fallows (one grower in each). Some growers reported applying up to 6 glyphosate applications per phase. These were recorded in GR cotton and winter fallows. Irrigated and non-irrigated GR cotton had the highest number of glyphosate applications with means of 3.2 and 3.0, respectively, and this was followed by the summer fallow which averaged 2.8 glyphosate applications. Irrigated GR cotton had the highest average number of applications to control glyphosate survivors (1.2) although some growers used five options to control glyphosate survivors in summer and winter fallows.

Overall risk

The combined total risk scores for each phase ($R_{T_{ij}}$) are listed in Table 6. The weed species and phase with the highest risk was *B. eruciformis* in non-irrigated GR cotton (12.3) and summer fallow (12.0). This was followed by *C. bonariensis* also in non-irrigated GR cotton (11.5) and summer fallow (11.2). When species present and management practices were combined for individual responses, *C. bonariensis* in summer fallow and other winter crops had very high risk situations (0–30). *S. oleraceus* had

Table 5. Management risk scores by phase for number of glyphosate applications, control of survivors of glyphosate applications, use of alternatives to glyphosate and the total management risk score (R_{M_j})

Scores are means followed by range in parentheses

Phase	No. of responses	Glyphosate applications (G_j)	Survivor control (C_j)	Number of alternatives (A_j)	Total (R_{M_j})
Summer fallow	31	2.8 (0–5)	0.7 (0–5)	1.1 (0–3)	1.5 (0–5)
Non-irrigated GR cotton	11	3.0 (2–6)	1.0 (0–3)	1.3 (0–7)	1.5 (0–4)
Non-irrigated non-GR cotton	1	2	1	0.5 (0–1)	1.4
Winter fallow	30	2.1 (0–6)	0.6 (0–5)	0.5 (0–3)	1.2 (0–5)
Irrigated GR cotton	22	3.2 (2–6)	1.2 (0–4)	1.1 (0–4)	1.1 (0–4)
Other winter crop	22	1.6 (0–4)	0.5 (0–3)	1.1 (0–3)	0.8 (0–4)
Sorghum	22	2.0 (0–5)	0.5 (0–2)	1.6 (0–4)	0.6 (0–4)
Wheat	32	1.3 (0–4)	0.5 (0–3)	0.9 (0–3)	0.5 (0–4)
Barley	14	1.0 (0–3)	0.3 (0–1)	0.9 (0–3)	0.5 (0–3)
Other summer crop	11	1.2 (0–3)	0.6 (0–2)	0.7 (0–3)	0.4 (0–1)
Irrigated non-GR cotton	7	1.3 (0–2)	0.6 (0–2)	1.6 (0–6)	0.2 (0–2)

Table 6. Mean total risk scores ($R_{T_{ij}}$) for highest risk weeds and phases

Maximum individual risk scores are indicated in parentheses (all individual risk scores had minimum values of zero). Single dashes in predicted total risk scores indicate an unlikely combination for which no estimate is made

Species	Summer fallow	Non-irrigated GR cotton	Non-irrigated non-GR cotton	Winter fallow	Irrigated GR cotton	Other winter crop	Sorghum	Wheat	Barley	Other summer crop	Irrigated non-GR cotton
				Predicted total risk (mean management risk per phase × species risk)							
Brachiaria eruciformis	12	12.3	11.4	9.8	9.2	–	5.2	–	–	3	1.7
Conyza bonariensis	11.2 (30)	11.5 (22)	10.6 (14)	9.2 (23)	8.6 (21)	6.4 (30)	4.9 (17)	3.7	3.8 (16)	2.8 (6)	1.6 (14)
Urochloa panicoides	10.7 (22)	10.9 (21)	10.1	8.7 (11)[A]	8.1 (21)	6.1 (22)[A]	4.6 (16)	–	–	2.7 (7)	1.5 (2)
Chloris virgata	10.4 (28)	10.6 (7)	9.9	8.5 (15)[A]	7.9	–	4.5 (6)	–	–	2.6	1.5
Sonchus oleraceus	10.1 (28)	10.4 (7)	9.6	8.3 (28)	7.7 (14)	5.8 (27)	4.4 (6)	3.4 (14)	3.4 (17)	2.6 (1)	1.4
Echinochloa colona	10.1 (27)	10.4 (27)	9.6	8.3 (21)[A]	7.7 (27)	5.8 (21)[A]	4.4 (15)	–	3.4 (17)[A]	2.6 (7)	1.4 (2)
Eleusine indica	9.3	9.5	8.8	–	7.1	–	4	–	–	2.3	1.3
Phalaris paradoxa	9.3	–	–	7.6	–	5.3	–	3.1	3.1	–	–
Hordeum sp.	9.3	–	–	7.6	–	5.3	–	3.1	3.1	–	–
Lolium rigidum	9.3 (7)	–	–	7.6	–	5.3 (12)	–	3.1	3.1	–	–
Dactyloctenium radulans	8.7 (24)	9	8.3	7.1	6.7	5	3.8	2.9	2.9	2.2	1.2
Digitaria ciliaris	8.7 (24)	9	8.3	7.1 (12)	6.7	5	3.8 (13)	2.9	2.9	2.2	1.2
Chloris truncata	8.7 (24)	9.0 (6)	8.3	7.1 (9)	6.7 (12)	5	3.8 (5)	2.9	2.9	2.2	1.2 (1)

[A] *U. panicoides*, *E. colona* and *C. virgata* are not generally considered to be winter weeds; however, some growers indicated they were present in some winter crops and fallow.

very high risk situations in summer and winter fallow (0–28), as did *C. virgata* and *E. colona* in summer fallow (0–28). The range of grower individual scores varied. For example, the industry mean for *C. bonariensis* in other winter crops was only 6.4 compared with an individual response of 30. This highlights the wide range of management practices adopted by growers throughout the industry. The summer fallow in general had higher risks for all species when compared with other phases. The use of alternatives and survivor control in the summer fallow was generally less than both irrigated and non-irrigated GR cotton.

Discussion

Species at risk

All species with confirmed glyphosate resistance in the sub-tropical cropping region of Australia, *C. bonarensis*, *E. colona*, *U. panicooides*, *L. rigidum* and *C. truncata*, were present within the top 10 species and had species risk scores 5.9 or above. This is a good indication of the usefulness of our risk assessment process.

There are five species in the top 10 that are currently not confirmed with glyphosate resistance in this region – *B. eruciformis*, *S. oleraceus*, *C. virgata*, *E. indica* and *Hordeum* species. *B. eruciformis* is common in central Queensland and is a high risk species for that region (Osten *et al*. 2007), but not found in the cotton-growing areas of southern Queensland and northern New South Wales. Our survey did not include central Queensland and thus no overall risk scores were calculated for that species.

S. oleraceus is a common weed across the sub-tropical cropping region and has the ability to germinate all year round. Although *S. oleraceus* has virtually no dormancy (Chauhan *et al*. 2006; Widderick *et al*. 2010), it is rarely present in the field in dense populations. Fallows infested with *S. oleraceus* are normally treated with mixtures of glyphosate and 2,4-D (Walker *et al*. 2005). This combination with different modes of action (Groups M and I) would be expected to reduce the risk of glyphosate resistance evolution. Thus, it is likely that the lower average population density and less exposure to glyphosate alone are reasons why glyphosate resistance has not yet been observed in this species.

C. virgata is a species that is a major problem in central Queensland (Osten 2008) but is increasing in prevalence in north-eastern Australian cropping systems. This species is not particularly susceptible to glyphosate and current practices may have led to a species shift rather than evolved resistance, though in both cases the effectiveness of glyphosate is reduced.

E. indica and *Dactyloctenium radulans* (R.Br.) Beauv. are both not widespread (Charles *et al*. 2004; Walker *et al*. 2005), and as a result are not considered major resistance issues for the industry as a whole. Even so, if present in the field, they require particular attention, particularly as populations of *E. indica* are glyphosate-resistant overseas.

The top 20 species are dominated by the annual grasses and members of the Asteraceae family. This is predominately due to their high seed production, which is a common characteristic of current resistant species. Therefore, these are the species that growers need to monitor closely.

Risks in crops and fallows

Within the diverse cropping systems of the Australian sub-tropical region, the high risk phases were non-irrigated GR cotton, summer fallow, and to a lesser extent irrigated GR cotton. This assessment confirmed previous perceived risks and again indicates the usefulness of our risk assessment process.

However, the overall risk was highly dependent on the weed species present in the different phases of the rotation. As an example, the average risk for 'other' winter crops was approximately half of that for summer fallow and non-irrigated GR cotton. However, when *C. bonariensis* was present in the 'other' winter crops phase, individual risks were the same as for the summer fallow (both 30), and higher than non-irrigated GR cotton (22).

Eleven of the 50 surveyed growers indicated that they grew non-irrigated GR cotton. In this phase, the risks for *C. bonariensis*, *U. panicoides* and *E. colona* were high. Currently there is only one confirmed resistant *E. colona* population in a non-irrigated GR cotton system (Werth *et al*. 2010), though the number of cases is likely to increase. It is concerning that there were growers, who indicated they did not control survivors of glyphosate application, despite the requirements to do so (Werth *et al*. 2008). These growers are likely to have thought that glyphosate provided sufficient control negating the need for further action. The individual responses of non-irrigated GR cotton growers did, however, indicate that they all used an alternative to glyphosate at some stage, and thus no grower in this survey relied on glyphosate only for weed control.

The risk scores for the summer fallow were consistently high. Glyphosate has been relied upon for weed control in the summer fallow phase for several years. It is unclear if the survivor control in the fallows were separate applications specifically for controlling glyphosate survivors, or if they were included in other herbicide applications. Tank-mix partners such as 2,4-D, picloram and MCPA do not act as alternatives for grass control. Therefore, research now concentrates on strategic use of residual herbicides and tillage to reduce the reliance on glyphosate. In fallow situations, Group A herbicides (ACCase inhibitors) are starting to be used. These herbicides have a high resistance risk, and as a result, the impact of their increased use in fallow needs to be examined thoroughly before promotion to growers.

Conclusions

Overall, the individual and average risks for glyphosate resistance in cotton systems of sub-tropical Australia vary considerably. This highlights the importance for growers to individually assess their own situation in terms of species present and their management practices. Our risk assessment framework will enable growers to tailor their weed management to focus on those species that are at a high risk of evolving resistance. They will need to use effective alternatives to glyphosate, which when targeted at their at-risk weed species will help to ensure the long-term sustainability of glyphosate.

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