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Susceptibility of fleabane (*Conyza bonariensis*) biotypes to glyphosate in northern cotton farming systems of Australia

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ABSTRACT

The rapid emergence of herbicide-resistant weeds has further added complexity to the management of weeds in Australian cotton farming systems. Fleabane (*Conyza bonariensis* L) has been identified by the cotton industry as being potentially resistant or at risk of developing resistance. Thirty-seven *C. bonariensis* populations were collected in the 2014-2015 cotton season from 9 cotton farming regions in Queensland and New South Wales. Glasshouse screening trials showed that 27 populations (73%) expressed resistance to glyphosate salt at rate of 0.68 kg a.i ha^{-1} applied at the rosette stage. Another 9 populations (24%) were categorised as developing resistance. A dose response study of 4 selected resistant populations using glyphosate rate of 0, 0.34, 0.68, 1.36, 2.72, and 5.44 Kg a.i ha^{-1} showed a high level of Resistance Index (RI) ranging from 3.5 to 7.0 and the dose-response model revealed that 3 to 4 times more glyphosate is required to control resistant *C. bonariensis* populations. These results further confirm the frequent reports of reduced efficacy of glyphosate against fleabane in northern cotton farming systems of Australia. To reduce the risk of resistance other non-glyphosate tactics are needed in the weed management toolbox.

Introduction

Cotton (*Gossypium hirsutum*) is an economically important crop in Australia, producing between two to five million bales each year with an export value of almost $3 billion in 2011–2012 (Cotton Australia 2017). Weeds are detrimental to cotton production and heavy infestations can lead to a significant yield reduction (Dogan et al. 2014; Morgan et al. 2001; Oerke, 2006). Fleabane (*Conyza bonariensis* L.) is an annual or short-lived perennial weed species (Wu, 2007). *Conyza spp.* infestation at emergence stage of cotton can reduce lint yield by 46% (Steckel and Gwathmey, 2009). It is also commonly found in non-cropped areas and tolerates a wide range of climates and

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habitats (Michael, 1977; Wu, 2007). In recent years, it has now become a major weed problem throughout most of cotton growing regions (Manalil et al. 2017). The change in farming practices to zero till systems results in a greater retention of soil moisture in no-tilled fields, and reduced reliance on soil-applied residual herbicides, contributes to the success of *C. bonariensis* in Australian cropping system (Walker and Robinson, 2008; Wicks et al. 2000).

Glyphosate-resistant cotton was introduced to Australian cotton farming systems in the 2000-2001 seasons. Since then, it has been widely adopted as a flexible weed control option and now encapsulates 99% of all cotton areas sown. The rapid adoption of this technology has coincided with a shift toward minimum and no till farming (Givens et al. 2009). The prevalence of this system has also resulted in a reduction in non-chemical weed control tactics such as tillage and hand chipping, with increased heavy reliance on glyphosate for weed control. Indeed, many growers are adopting a glyphosate only approach to weed control or limiting the use of other herbicides and non-herbicide tactics (Young, 2006). A move away from the use of residual and pre-emergent herbicides is placing additional pressure on glyphosate to do all the heavy lifting for weed control. Such over-reliance on glyphosate for weed control during the past decade has resulted in adverse repercussions including weed species shifts and the evolution of glyphosate-resistant weeds (Culpepper, 2006; Powles and Yu, 2010; Wilson et al. 2007).

Herbicide resistance in weed populations has spread across the cropping systems in Australia and includes resistance to glyphosate in *C. bonariensis* populations (Walker and Robinson, 2008). Herbicide resistance is an evolutionary process where survival and reproduction of individuals with resistance alleles in a population are enriched in the presence and absence of the herbicides (Powles and Yu, 2010). The resistance mechanisms of weed populations are greatly influenced by genetic (reproduction and mating system, population size, number of generations) and by environmental conditions (Jasieniuk et al. 1996; Maxwell and Mortimer, 1994; Vila-Aiub et al. 2005). Whilst time consuming and expensive, identifying the underlying levels of resistance in weeds species across large areas is important in better understanding herbicide resistance and designing best management practices (Broster et al. 2011). Information gained from surveys can aid in the planning of herbicide resistance research and extension for specific areas (Llewellyn and Powles, 2001). Herbicide resistance surveys of any weed species are also useful for future prediction of resistance development and to understand survival in response to herbicides (Goh et al. 2015). The status of glyphosate resistance and population dynamics of *C. bonariensis* is important for providing useful recommendations to growers. This study was conducted to evaluate

the current level of glyphosate resistant in *C. bonariensis,* infested in northern cotton farming systems of Australia.

Materials and Methods

Seeds collection and sowing

In the 2014–15 cotton season a survey was conducted across 9 cotton farming regions in Queensland and NSW (Figure 1) and seed samples of 37 populations were collected. Seeds of these populations were sown on the soil surface of plastic pots (25 cm in diameter) pre-filled with potting mix and lightly covered with field soil. The pots were initially hand-watered, covered with paper towel, and maintained in a glasshouse. The paper towel was removed once fleabane has emerged. Fleabane seedlings from each pot were transplanted to trays filled with similar type of potting mix (6 alternating spots on the tray) at two to four-leaf stage on 8th November 2016. Each population had 18 experimental units in three replications.

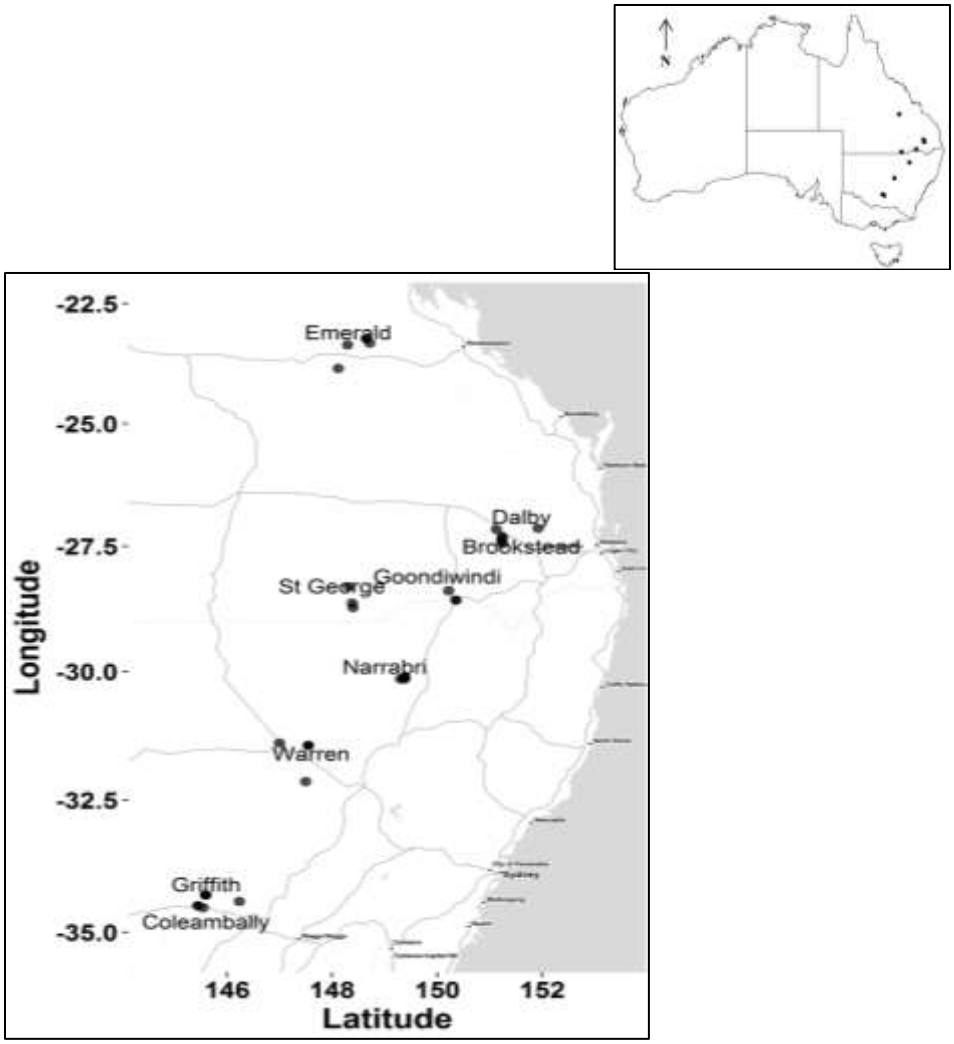

Figure 1. *C. bonariensis* sample location from cotton farms for the 2014-15 survey

Preliminary screening and measurements

Fleabane populations were sprayed at rosette stage (8–10 cm diameter, 8–10 expanded leaves) with 0.68 kg a. i ha^{-1} of glyphosate (using glyphosate-540) (Walker et al. 2011). The herbicide were applied on 24th November 2016 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 on 24th (before spray) and recommenced on 25th November 2016. Three trays were arranged in a completely randomized 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 >20% after spraying with 0.68 kg a. i ha^{-1} of glyphosate were considered ''resistant", those with between 10% and 20% survival ''intermediate or developing resistance" and those with plant death and necrosis >90% or plant survival less than 10% were considered as "susceptible' (Broster et al. 2011).

Dose–response assay in selected populations

Based on initial screening, 5 populations of *C. bonariensis* were selected. A total of six different herbicide rates (0, 0.34, 0.68, 1.36, 2.72 and 5.44 kg a. i ha^{-1} of glyphosate) were sprayed on these biotypes separately. The populations were sprayed in the automated spray cabinet as described above. The biomass of surviving plants of each population under different herbicide rates were assessed at 28 days after application.

Model fitting and statistical approaches

Data were analysed using R software (R Core Team 2017) operating in RStudio. Extensive use was made of R package *drc* (Ritz et al. 2015). The five numbers summary was produced by vertical box-plots. Data normality and distribution was verified by Q-Q plot and Shapiro-Wilk normality test. A binary logic regression model: glm (formula = plant survived~population, family = binomial, link = "logit") with Fisher scoring was used to analyse the response variable (plant survived) of preliminary screening. The log-logistic non-linear dose response model (Seefeldt et al. 1995) was used to determine the response *Y* (% of biomass control) to glyphosate dose (*X*) according to the following formula:

$$Y = c + \frac{d - c}{1 + \exp\{b(\log(x) - \log(LD_{50}))\}}$$

In this equation, *d* and *c* is the upper and lower responses accordingly. The upper limit *d* corresponds to the mean response of the control and the lower limit *c* is the mean response at very high doses. LD_{50} is the herbicide dose required to reduce 50% of plant biomass and *b* is the slope of the curve around LD_{50}. The Resistance Index (RI) was determined by dividing the LD_{50} value of each resistant biotype by the LD_{50} of the susceptible biotype. The LD_{50} and RI values were separated by Fishers' least significant difference (LSD) ($P = 0.05$). A linear model was also checked to verify the acceptance of log-logistic non-linear model through Akaike Information Criterion (AIC) test (linear-model; 867 < log-logistic model; 614). The goodness of fit in regression was also assessed by R^2.

Results and Discussion

Preliminary screening and measurements

Among the 37 *C. bonariensis* populations tested with glyphosate at 0.68 kg a.i ha^{-1}, a total of 27 tested populations were resistant and 9 populations were categorised as developing resistance (Figure 2). Only one population, the 1F3 from Emerald Central Queensland, was quantified as susceptible (Table 1). All tested populations collected from Brookstead, Dalby, Goondiwindi, St George and Coleambally were resistant. Our findings are in general agreement with the observations that glyphosate-resistant fleabane populations are wide spread in Australia (Walker and Robinson, 2008; Wicks et al. 2000) and overseas (Travlos and Chachalis, 2010; Urbano et al. 2007). Herbicide resistance is dynamic and the current research demonstrated a wide geographical distribution of *C. bonariensis* that is not controlled by a glyphosate test rate of 0.68 kg a. i ha^{-1}. However, it is also possible that both glyphosate resistance and susceptible *C. bonariensis* individuals can be present in adjacent areas. So, further survey and corresponding biological resistance testing needs to be considered. The question then arises, as to whether these glyphosate resistant *C. bonariensis* populations are from a single source that has spread or whether glyphosate resistance developing in separate locations as unique events. The exploration of this question will be an area of future study, involving an examination of the physiological and genetic basis for the observed resistance.

Figure 2. *C. bonariensis* populations before (top slide) and after (bottom slide) application of glyphosate in the glasshouse.

Dose–response assay in selected populations

The response of each *C. bonariensis* biotype to an increasing dose of glyphosate was fitted to a log-logistic non-linear model (Figure 3), which confirmed significant (*P*= 0.05) differences in biomass reduction in response to glyphosate especially, at 0.34 kg a. i ha^{-1}, and 0.68 kg a. i ha^{-1}. At the test rate of glyphosate (0.68 kg a. i ha^{-1}), less than 50% of plant biomass was controlled in 4 resistant populations.

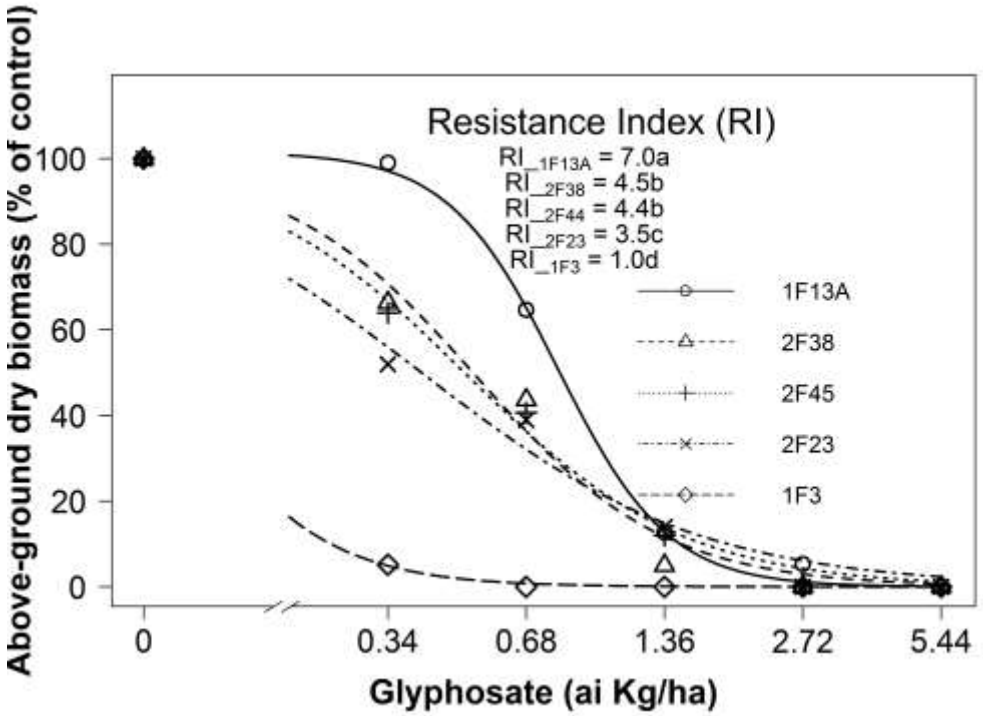

Figure 3. Dose–response assay of glyphosate-resistant and susceptible populations of *C. bonariensis* treated at the rosette stage with five different glyphosate doses. Lines describe the predicted survival responses according to the equation reported in the materials and methods section. Symbols shown are the original means (n = 6) of biomass (% of control) of the populations. Data were pooled of three experimental units and six observational units. In the inset, for each biotype the resistance index (RI) and statistical significance (different letters represent values significantly different at *P*=0.05 based on Fisher's LSD) are reported.

The untreated plants of population 1F13A developed more biomass, evident by the *d* value estimated for the growth parameters (Table 2). This population kept more biomass production than other resistant populations until it received a glyphosate rate of 1.6 kg a. i ha^{-1}. Here, all populations showed similar patterns in the herbicide dose-response curve. This indicates that herbicide dose is important in weed suppression, with higher doses inducing greater inhibition for resistant weed populations. A high dose well beyond the threshold concentration was required to produce an effect. The trend line for resistant populations also indicating that glyphosate lost its inhibition potency for these populations. Plants of these populations may have enough time to recover from the toxicity of glyphosate salt. However, other internal and external factors including type of exposure, exposed species, or plant growth stage may be involved for recovery such that a herbicide is not always and consistently responsible for resistance under all circumstances. Here RI values of resistant populations were varied; therefore we can assume that different internal mechanisms were expressed by different resistant populations.

Table 1. Resistance levels for the screened glyphosate (R-resistant >50% survival; DR-developing resistant <50 %-> 20% survival and S- susceptible <20% survival).

Location	Number of population tested	ID	Plant survived (%)	Status
Emerald	6	1F1	83	DR
		1F2	100	R
		1F3	33	S
		1F4	44	DR
		1F6	44	DR
		1F7	100	R
Dalby	4	1F9	78	R
		1F10	94	R
		1F11	78	R
		1F12B	88	R
Brookstead	4	1F13A	100	R
		1F13B	94	R
		1F14	94	R
		1F16	88	R
Goondiwindi	3	1F20	100	R
		1F21	88	R
		1F21B	94	R
St-George	2	2F23	100	R
		1F25	100	R
Narrabri	5	1F31	88	R
		1F34	94	R
		2F35	94	R
		1F36	50	DR
		1F37	50	DR
Warren	5	1F38	50	DR
		2F38	50	DR
		1F41	100	R
		1F42	83	R
		2F42	72	R
Coleambally	3	1F44	100	R
		2F45	83	R
		2F45B	100	R
Griffith	5	2F46	50	DR
		2F47	50	DR
		2F48	88	R
		2F49	88	R
		2F50	88	R
Total tested 37			**Total 73% R and 24% D-R**	

Significant differences between populations within a location can also be found in *C. bonariensis* resistant biotypes. Dinelli et al. (2008) reported that RI differs between biotypes of the same genus of weeds, even within the same populations. The role of such resistant mutations needs to be clarified for geographic distribution of resistant populations of *C. bonariensis*. Whilst our current research is not focused on this question, we confirmed that a high proportion of the *C. bonariensis*

population is currently resistant to glyphosate and that the relative ranking of these resistant biotypes may differ.

Table 2. Estimated regression parameters (Equation 1) from dose-response study on the basis of biomass control of *C. bonariensis* treated with glyphosate at rosette stage.

Parameter estimates (±SE)					
Population	Category	*b*	*d*	LD_{50} (Kg ha^{-1} a.i)	95% CI
1F13A	R	3.629	101.500	0.7983 (0.038)	0.723-0.873
2F38	R	2.116	98.620	0.5278 (0.041)	0.041-0.445
2F45	R	1.836	99.300	0.5033 (0.042)	0.041-0.420
2F23	R	1.434	99.410	0.4060 (0.043)	0.319-0.492
1F3	S	2.689	100.00	0.1130 (0.285)	-0.456-0.682

b, relative slop around $LD_{50,}$ where LD_{50} are effective doses (Kg ha^{-1} a.i) of causing 50% biomass reduction in shoot dry weights. *d,* upper limit of the response, *CI* is confidence interval.

From the dose response model, the observed resistance index (RI) was 3.5 to 7, that is higher than previously calculated in other populations of this species in Australia by Walker and Robinson (2008). A glyphosate rate of 0.68 kg a.i ha^{-1} (X) provided complete control of the susceptible (S) population, while 5.44 kg a.i ha^{-1} (4X) was needed to provide full control of resistant populations 2F38 and 2F45. Glyphosate rates needed to be increased by eight times the test rate to control some resistant populations in comparison with the S population. A glyphosate rate of 0.11 kg a.i ha^{-1} caused 50% biomass reductions in the susceptible populations 1F3, whereas the estimated dose for 50% reduction of 2F23 for 0.406 Kg ha^{-1}, 0.503 Kg ha^{-1} for 2F45, 0.527 Kg ha^{-1} for 2F38 and 0.798 Kg ha^{-1} for 1F13A (Table 2). The biologically effective dose can be utilised to ensure profit maximisation and to reduce the amount of herbicide applied into the environment (Knezevic et al. 1998). Here, despite exposure to a high dose of herbicide, plants of 1F13A population still survived, suggesting that individuals within this population had already evolved resistance. The same mode of action is likely to result in reduced control of *C. bonariensis* plants in the field and high dose rates may not be cost-effective for growers. Typically, high rates will select faster because the selection pressure is stronger. Conversely, low rates can select for weak resistance mechanisms and can result in resistant populations with more complex mixtures of resistance mechanisms (Preston et al. 2018). Herbicides are used to provide effective weed control and dose could be selected to either control the weed, or reduce its growth depending on weed species, and growth stage of *C. bonariensis.*

Conclusion

In conclusion, our survey revealed that a high proportion of *C. bonariensis* populations have resistance to glyphosate in key cotton growing regions of Australia. The use of herbicide with the same modes of action, without survivor control, may accelerate resistance build up and increase the difficulty of resistance management. There are benefits to be derived from growers controlling glyphosate survivors in their paddock and diversifying weed management practices to avoid selecting more resistant *C. bonariensis* populations. Tactics may include using pre- and post-emergence herbicides with different modes of action, greater use of soil-applied residual herbicides, reducing weed banks by other cultural practices and strategic tillage. Furthermore, there is considerable opportunity to improve control through research to determine the physiological and ecological mechanism of glyphosate resistance in *C. bonariensis*, as well as the pattern of inheritance.

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Conflicts of Interest

The authors declare no conflicts of interest.

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