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Resistance of flaxleaf fleabane (*Conyza bonariensis* (L.) Cronquist) to glyphosate

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Abstract

Background: Resistance to glyphosate has been reported in flaxleaf fleabane populations within a number of grain growing areas in southern Queensland and northern New South Wales and other cropping regions across Australia.

Results: To investigate the processes contributing to the evolution and spread of glyphosate-resistant flaxleaf fleabane, the resistant populations tested in dose-response experiments varied in their responses to glyphosate from two to eight times the recommended rate. The different dose responses obtained may indicate different mechanisms of resistance. This can possibly be attributed to an independent evolution of resistance.

Conclusion: The results demonstrate that glyphosate resistance is widespread across Australia and has likely evolved multiple times as well as dispersing by seeds.

Keywords: Glyphosate, Flaxleaf fleabane, *Conyza bonariensis*, Herbicide resistance

Introduction

Flaxleaf fleabane (*Conyza bonariensis*) is a summer-growing weed species belonging to the family Asteraceae (Wu, 2009). Flaxleaf fleabane is an important weed species in agriculture and environmental systems worldwide (Randall, 2017) and is distributed broadly over the warmer environments (TERZİOĞLU & ANŞİN, 2001). Flaxleaf fleabane has recently become a serious problem in a number of grain growing areas in southern Queensland, northern New South Wales and other cropping regions across Australia Wu, Walker (Wu et al., 2006); (Walker & Robinson, 2008); Owen, Owen (Owen et al., 2009). The continual application of glyphosate [N-(phosphonomethyl) glycine] for flaxleaf fleabane control has resulted in the evolution of resistance to this herbicide (Walker & Robinson, 2008). The evolution of resistance in numerous weed species worldwide to the commonly used, highly effective, systemic herbicide glyphosate, which is non-selective and wide spectrum, is of particular concern because this herbicide is important for the sustainability of grain cropping in Australia (Wakelin & Preston, 2006). The first report of glyphosate-resistant populations in flaxleaf fleabane was in South Africa in 2003 according to Moreira et al. (Moreira et al., 2007), and since then, populations resistant to glyphosate have been reported in other countries including the USA, Spain, Colombia, and Brazil. McGillion and Storrie (McGillion & Storrie, 2006) reported that flaxleaf fleabane is one of the most common herbicide-resistant weed species overseas. As a result of the use of glyphosate in summer fallow operations, glyphosate-resistant populations of flaxleaf fleabane have begun to appear in Australia, with the first resistant population identified in 2011 (Aves et al., 2017; Walker et al., 2011).

In Australia, a range of responses has been found to glyphosate among populations of flaxleaf fleabane. Walker et al. (Walker et al., 2011) reported that populations from cropping paddocks seem to be more resistant to glyphosate than those from pastures or non-agricultural sites. Glyphosate resistance was initially confirmed in eight populations of flaxleaf fleabane, from northern NSW and southern Queensland in Australia; since then, the number of resistant populations identified has rapidly increased (Walker et al., 2011). However, the evolution of glyphosate resistance in flaxleaf fleabane is poorly understood. The main objective of this study

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Table 1 Names, locations, and response to glyphosate of 15 populations of flaxleaf fleabane selected for the dose-response experiment

Location	Study label	Response to glyphosate
SNSW	FLE01	Resistant
SNSW	FLE02	Resistant
SNSW	FLE08	Resistant
SNSW	FLE23	Resistant
SA	FB 02C	Resistant
SA	FB 03C	Resistant
SA	FB 05C	Resistant
SA	FB 06C	Resistant
SA	FB 07C	Resistant
NNSW	NNSW04	Resistant
SEQSLD	SEQLD05	Resistant
VIC	FB01	Susceptible
NNSW	NNSW14	Resistant
SEQSLD	SEQLD3B	Resistant
NNSW	NNSW06	Resistant

Abbreviations: *SNSW* southern New South Wales, *SA* South Australia, *NNSW* northern New South Wales, *SEQSLD* south-eastern Queensland, *VIC* Victoria

was to determine the extent of flaxleaf fleabane resistance to glyphosate, using a dose-response study.

Material and methods

Plant materials

Fifteen populations of flaxleaf fleabane were collected from roadside survey across Australia by Malone et al. (Malone et al., 2012), (Table 1).

Seed germination and plant growth

Approximately 0.2 g of seeds from each population were sown in separate trays (30 by 20 by 10 cm) containing coca peat soil in a glasshouse. Seeds were sown directly onto the soil surface and uniformly irrigated as required until an appropriate number of seedlings for each population had emerged. For the dose-response experiment, seedlings were transplanted into standard pots (with 15 cm diameter) at a density of five to 12 seedlings/pot. The plants were maintained under usual growing conditions (outdoor as they were planted in March 2012) throughout the growing period and watered with a mist spray once or twice a day as required, until herbicide application as described below.

Herbicide application

Dose-response experiments were performed on 15 populations of flaxleaf fleabane, one susceptible and 14 resistant as highlighted in Table 1. After 4 weeks of germination, when

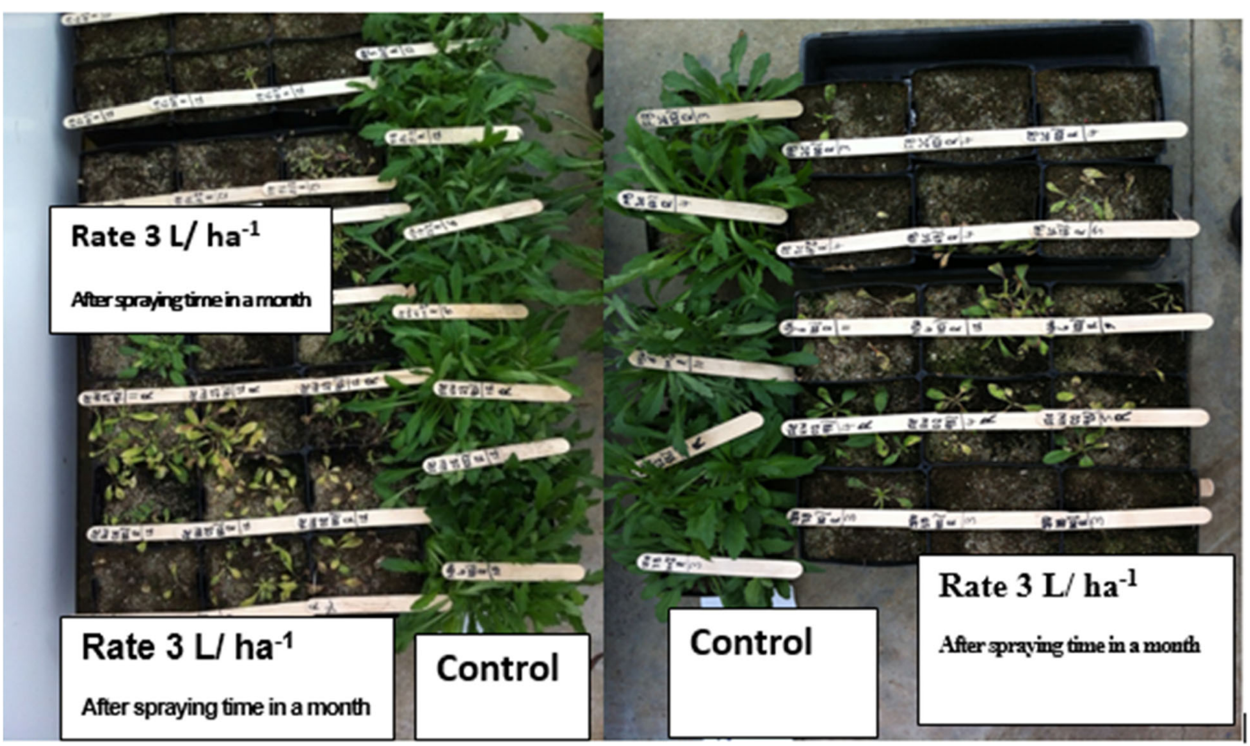

Fig. 1 Response of the fourteen resistant populations of flaxleaf fleabane 4 weeks after treatment with the highest recommended rate of glyphosate (1500 g a.i. ha^{-1} glyphosate)

the plants were at six to eight leaf stage, they were treated with glyphosate. The experiments were set up in a randomized complete block design with three replicates and six rates. The doses applied to resistant populations were 0-, 1-, 2-, 4-, 6- and 8-fold of field rate (field rate 750 g a.i. ha^{-1}), while to the susceptible population, doses applied were 0-, 0.25-, 0.5-, 1-, 2- and 4-fold of field rate of glyphosate (touchdown high tech 500 g/L Monsanto, Melbourne, Victoria, Australia). Adjuvant BS 1000 was added at 0.2% v/v. The lowest and highest recommended doses of glyphosate for flaxleaf fleabane are 750 and 1500 g a.i. ha^{-1}, respectively.

The glyphosate treatment was applied to the plants using a moving-boom laboratory twin nozzle sprayer sited 40 cm above the seedlings, with a water volume of 110 L ha^{-1} at a pressure of 250 kPa. A moving belt holds the nozzle at a speed of 1 ms^{-1}. The dose-response experiment was carried out at the Waite Campus of the University of Adelaide, South Australia, after 3–4 months from the seedling transplantation in March 2012.

Plants treated with glyphosate were held in the spray treatment laboratory for 3 h to allow the herbicide to dry before being returned to the glasshouse or outdoors. Plants were watered as required. Four weeks after the application of glyphosate, plants were recorded as dead (susceptible) or alive (resistant). Any plants with green tissue, even if pale, were considered resistant, as they would recover later.

Data analysis

Dose-response data were subjected to probit analysis version 1.63 (US Department of Agriculture, www.ars.usda.gov/Services/docs.htm?docid=11248) to determine the herbicide dose-response relationships from the percentage of surviving plants. LC_{50} (the herbicide dose causing 50% plant mortality) was calculated for each population. The difference in resistance levels between populations was determined by comparing the ratio of LC_{50} for each resistant population compared to the susceptible population.

Results

The fifteen populations of flaxleaf fleabane showed variation in survival rates to glyphosate applications at the highest recommended dose 1500 g a.i. ha^{-1} in a previous study (Malone et al., 2012) and, hence, were chosen for this study to determine the level of resistance to glyphosate.

Fourteen populations of flaxleaf fleabane resistant to the highest recommended dose of glyphosate (1500 g a.i. ha^{-1}) were confirmed for resistance (Fig. 1). In comparison, the

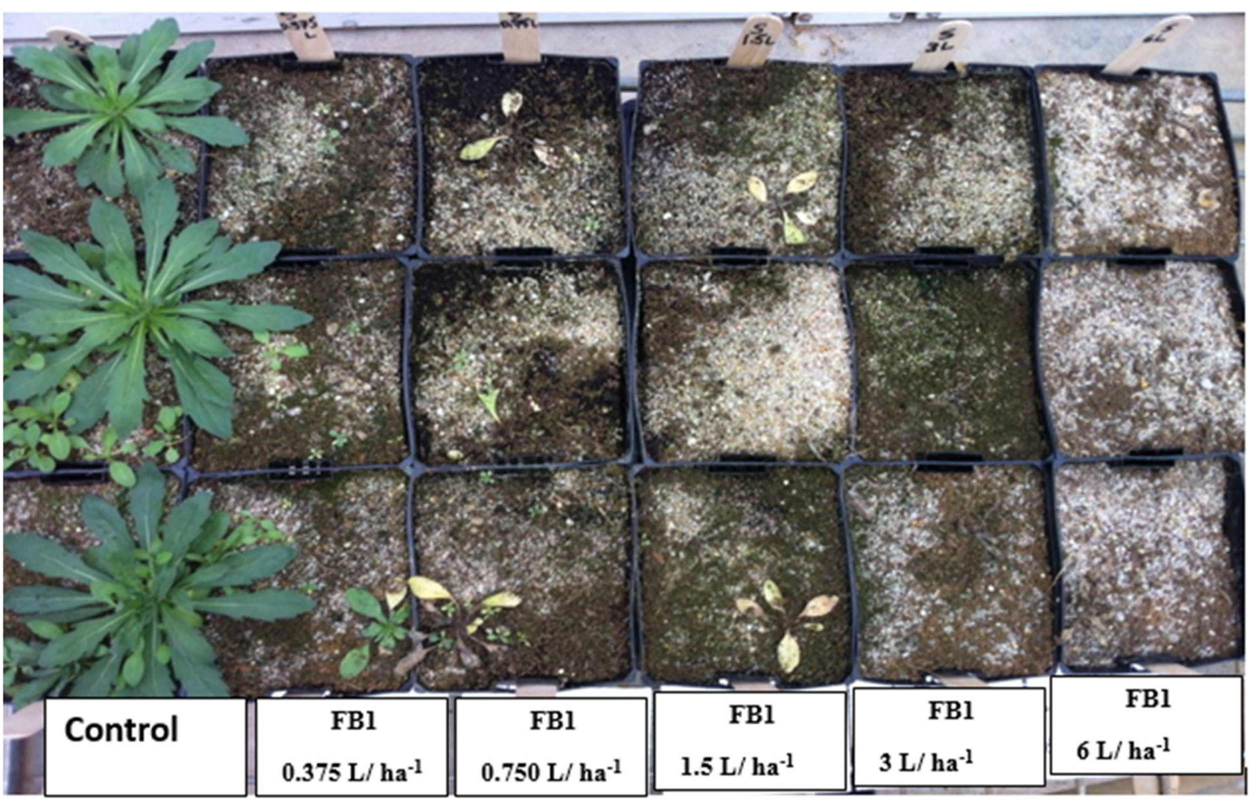

Fig. 2 Response of the susceptible population (FB1) of flaxleaf fleabane 4 weeks after treatment with the six rate of glyphosate (0, 375, 750, 1500, 3000 and 6000 g a.i. ha^{-1})

response of the susceptible population (FB1) of flaxleaf fleabane to six rates of glyphosate (0, 187.5, 375, 750, 1500 and 3000 g a.i. ha^{-1}) is shown in Fig. 2. The susceptible population (FB1) was totally killed when treated with the lowest recommended dose of glyphosate (750 g a.i. ha^{-1}) and the higher doses. It was also controlled well (75%) at the rate of 375 g a.i. ha^{-1}, which is below the recommended rates. The survival rate of six resistant populations and one susceptible population of flaxleaf fleabane, following treatment with different rates of glyphosate, is shown in Fig. 3.

The responses of the resistant populations to glyphosate varied and were grouped into one of six responses arbitrarily based on LC_{50} values and representatives of each response are illustrated.

Among the resistant populations, the population that was most sensitive to the highest recommended rate of glyphosate (1500 g a.i. ha^{-1}) was FB5c, followed by NNSW4. Populations Fle01, Fle23, and NNSW14 were the most resistant to the recommended rates of glyphosate.

The LC_{50} values for glyphosate calculated from the probit analysis for the 15 flaxleaf fleabane populations (Table 2) showed that the population responses fell into seven groups [one susceptible (control) and six resistant].

The LC_{50} values for the FB6c, FB5c and SEQLD3B populations (group 1) were the lowest and varied from 440 to 511 g a.i. ha^{-1} and were 4.7- to 5.44-fold more resistant than the susceptible population (FB1). The LC_{50} values for the Fle08, SEQLD5, NNSW4 and NNSW6 populations (group 2) varied from 659 to 737 g a.i. ha^{-1} and were 7- to 7.9-fold more resistant than the susceptible control. The FB7c, FB3c and FB2c populations (group 3) formed a low moderate group, with LC_{50} values between 806 and 827 g a.i. ha^{-1}, giving 8.6- to 8.8-fold resistances. The LD_{50} for the NNSW14 population was 1035 g a.i. ha^{-1}, giving 11-fold resistances to glyphosate. Populations Fle02 and Fle23 (group 5) showed the second highest LC_{50} values, ranging from 1336 to 1404 g a.i. ha^{-1}, giving 14.2- to 14.9-fold resistance. The Fle01 population was the most resistant with LC_{50} of 2217 g a.i. ha^{-1}, giving 23.6-fold resistance at the seedling stage.

Discussion

Walker et al. (Walker et al., 2011) reported low levels of glyphosate resistance (two to sevenfold) in several populations of flaxleaf fleabane from northern NSW and southern Queensland. Many glyphosate-resistant populations have relatively low levels of resistance, such as

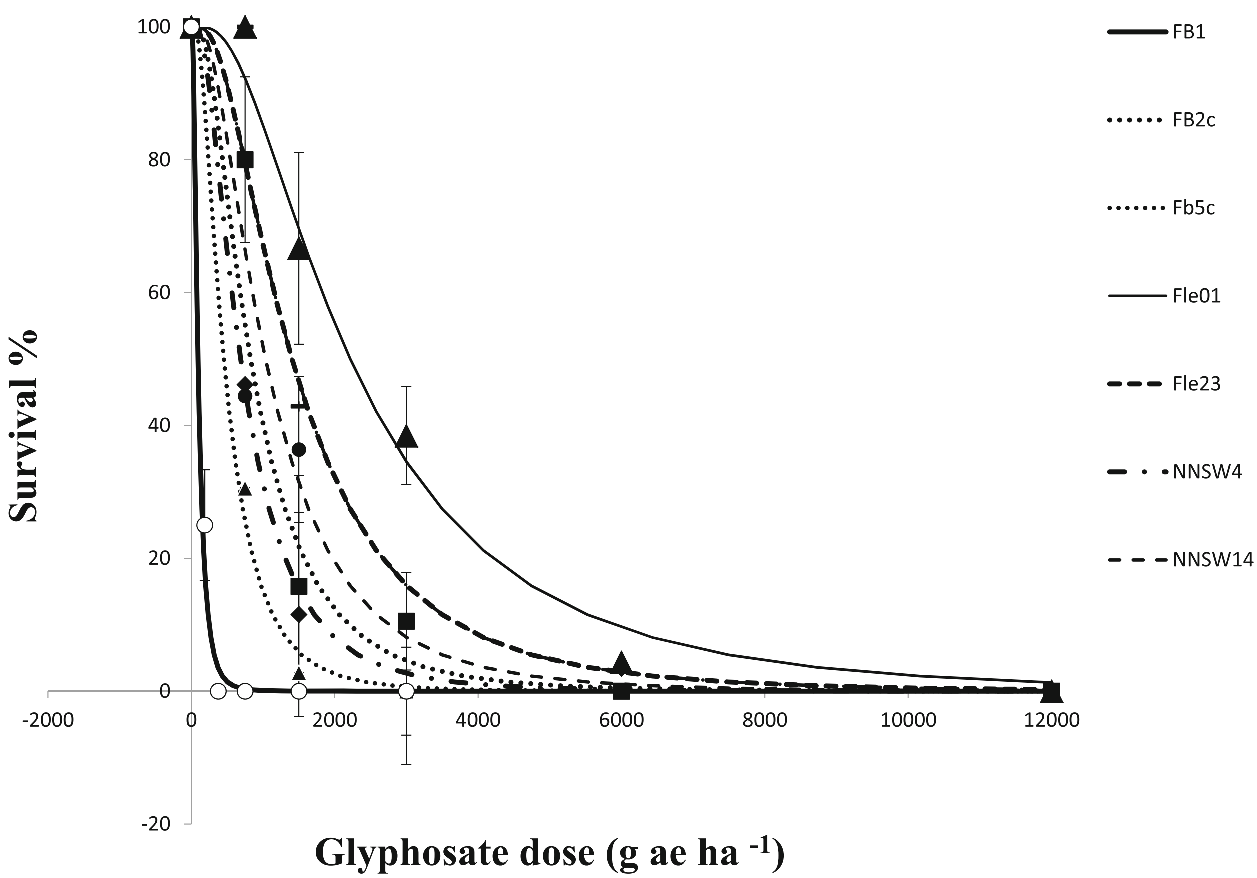

Fig. 3 Response of one susceptible and six resistant populations of flaxleaf fleabane to six rates of glyphosate. Each data point is the mean of three replicates ± standard error (SE)

Table 2 LC_{50} values of flaxleaf fleabane populations tested for glyphosate resistance. R/S is the ratio of the LC_{50}s of the resistant populations to that of the susceptible population (FB1)

POPULATION	LD_{50} (g ae ha-1)	R/S	GROUP*
FB1	94	-	Control
FB6C	440	4.7	1
FB5C	455	4.84	
SEQLD3B	511	5.44	
FLE08	659	7	2
SEQLD5	682	7.25	
NNSW4	688	7.32	
NNSW6	737	7.85	
FB7C	806	8.6	3
FB3C	813	8.65	
FB2C	827	8.8	
NNSW14	1035	11	4
FLE02	1336	14.2	5
FLE23	1404	14.9	
FLE01	2217	23.6	6

LC_{50} is the amount of glyphosate required to control at least 50% of the population; group* refers to populations that showed relatively similar patterns of response

annual ryegrass (Wakelin et al., 2004), awnless barnyard grass in Australia (Walker et al., 2011) and flaxleaf fleabane in Spain (Dinelli et al., 2008). On the other hand, there is more than 100-fold resistance in flaxleaf fleabane in IN, USA (Davis et al., 2008). These variations in the level of herbicide resistance may be the result of different resistance mechanisms (Preston et al., 2009). The four most resistant populations tested in this study were withstanding at rates of up to 12,000 g a.i. ha^{-1} glyphosate (eight times the recommended rate; Fig. 3). This result suggests that increasing herbicide dose will not improve weed control when resistance exists in a field. Therefore, alternative strategies will be required to control glyphosate-resistant flaxleaf fleabane populations. Furthermore, the relative significance of these differences among weed populations relates to the success of numerous management strategies in discontinuing or minimizing the dispersal of resistance to neighbouring regions, plantations and agricultural areas (Osuna et al., 2011). For example, in empirical studies on the long-distance spread of *Conyza canadensis* (Dauer et al., 2007) concluded that wind-dispersed weeds withstand any single practice of farm management as a practical controlling possibility for weed resistance to herbicides. Consequently, dispersal of weed seeds requires proactive management practices to prevent and/or at least minimize the growing invasions of herbicide resistance in undesirable weeds. Therefore, data on the mechanisms of resistance dispersion of *C. bonariensis* are required for the design of effective resistance management approaches for a cropping system.

Conclusion

The evolution of resistance in *C. bonariensis* worldwide to the commonly used, highly effective, systemic herbicide glyphosate, which is non-selective and wide spectrum, is of particular concern because this herbicide is important for the sustainability of grain cropping in Australia. The resistant populations tested in dose-response experiment varied in their responses to glyphosate from two to eight times the recommended rate. The different dose responses obtained may indicate different mechanisms of resistance. This can possibly be attributed to an independent evolution of resistance.

Abbreviations
LD50: The dose that more popular used in this case of foliar application; NNSW: Northern New South Wales; SA: South Australia; SE: Standard error; SEQSLD: South-eastern Queensland; SNSW: Southern New South Wales; V/V: Volume per volume; VIC: Victoria

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Authors' contributions
All work was done by MH under supervision and advice of CP and JM. MH was a major contributor in writing the manuscript. The authors read and approved the final manuscript.

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Competing interests
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