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 (TERZIOĞLU & 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...
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

| 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              |
| 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

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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 random-
ized 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 glypho-
sate (touchdown high tech 500 g/L Monsanto, Mel-
bourne, Victoria, Australia). Adjuvant BS 1000 was
added at 0.2% v/v. The lowest and highest recom-
mended 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 experi-
ment was carried out at the Waite Campus of the Univer-
sity 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 per-
centage 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 sus-
ceptible population.

Results
The fifteen populations of flaxleaf fleabane showed vari-
ation 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\(^{-1}\)).](image)
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
annual ryegrass (Wakelin et al., 2004), awnless barnyard grass in Australia (Walker et al., 2011) and flaxleaf flea-bane in Spain (Dinelli et al., 2008). On the other hand, there is more than 100-fold resistance in flaxleaf flea-bane 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.

| POPULATION | LD\(_{50}\) (g ae ha\(^{-1}\)) | R/S | GROUP* |
|------------|-------------------------------|-----|--------|
| FB1        | 94                            | -   | Control|
| FB6C       | 440                           | 4.7 |        |
| FB5C       | 455                           | 4.84| 1      |
| SEQLD3B    | 511                           | 5.44|        |
| FLE08      | 659                           | 7   |        |
| SEQLD5     | 682                           | 7.25| 2      |
| NNSW4      | 688                           | 7.32|        |
| NNSW6      | 737                           | 7.85|        |
| FB7C       | 806                           | 8.6 |        |
| FB3C       | 813                           | 8.65| 3      |
| 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.

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