Can herbicides affect seed dormancy and viability of flaxleaf fleabane (*Conyza bonariensis* (L.) Cronquist)

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Summary Flaxleaf fleabane (*Conyza bonariensis* (L.) Cronquist) has been considered a major weed in dryland cropping systems in Australia. Resistance in populations of *C. bonariensis* to glyphosate has also been confirmed. The weed is very difficult to control with any single herbicide application especially when herbicides are applied after the bolting stage. Anecdotal evidence suggests that some chemicals might affect fleabane seed viability and/or dormancy based on the observations that seeds collected from certain paddocks did not germinate. Glasshouse experiments were conducted to investigate whether applications of glyphosate and paraquat could sterilise the weed seeds and affect seed dormancy of fleabane. Results showed that both herbicides affected the seed viability and dormancy, depending on the application timing and the herbicide. The herbicide-induced dormancy decreased during storage under laboratory conditions.

Keywords Seed dormancy, viability, herbicides, germination.

INTRODUCTION

Conyza bonariensis (flaxleaf or hairy fleabane) is a cosmopolitan plant of temperate and sub-tropical climates, native to South America and a member of the Asteraceae (Everett 1990, Prieur-Richard *et al*. 2000). It invades a range of habitats including roadsides, wastelands, fencelines, fallows, cropping and pasture paddocks.

Research efforts have been concentrated on improved herbicide control efficiency particularly in cereal crops and fallows (Wu *et al*. 2008, 2010, Werth *et al*. 2010). It has been found that chemical control is more effective at the early rosette stage, and that control efficacy decreases with increasing plant age, especially after the bolting stage (Wu *et al.* 2007, Walker *et al.* 2012). A 'double-knock' technique, glyphosate + 2,4-D followed by paraquat ± diquat, has been widely adopted by growers to effectively control mature fleabane plants.

Flaxleaf fleabane has evolved resistance to glyphosate, with more than 60 resistant populations of fleabane confirmed (Walker *et al.* 2011, Preston 2016). When evaluating fleabane seed samples collected from the field for their glyphosate resistance status, it was found that some of these seed samples have little to no germination under ideal laboratory conditions (Wu, unpublished data), although Wu *et al.* (2007) has previously reported that fleabane seeds have no dormancy requirement for germination. It is suspected that heavy reliance on herbicides to control fleabane in the summer might have resulted in the loss of seed viability and/or induced dormancy. Herbicidal effects on seed viability have been previously documented in many weeds, including fleabane (Keenan *et al.* 2014). However, little information is available on the herbicidal impact on seed dormancy.

Seed dormancy refers to a physical and/or physiological block to the germination of viable seeds when they are imbibed under ideal germination conditions (Finch-Savage *et al.* 2006). It is a survival mechanism which contributes to the persistence of certain weed species, such as *Lolium rigidum* Gaudin (Owen *et al.* 2015). Seed dormancy is a highly complex trait, regulated by both genetic and environmental factors. Many environmental factors have strong influences on dormancy, especially during seed development and maturation, such as temperature, soil moisture, light quality and photoperiod (Gutterman *et al.* 2000, Swain *et al.* 2006). Farming practices such as nitrogen input could also induce dormancy (Luzuriaga *et al.* 2006). It has been documented that intensive cropping systems with heavy reliance of herbicides have altered weed seed biological traits, selecting for greater seed dormancy in such species as smooth barley (*Hordeum murinum* L. subsp. *glaucum* (Steud.) Tzvelev), ripgut brome (*Bromus diandrus* Roth) and *L. rigidum* (Fleet and Gill 2012, Kleemann and Gurjeet 2013, Owen *et al.* 2015). However, there is little information available about the direct linkages of herbicide applications with dormancy induction.

Glasshouse trials were established in 2014 to evaluate the impact of two most commonly used herbicides, glyphosate and paraquat, on fleabane seed

viability and dormancy. Flaxleaf fleabane plants were sprayed at two growth stages (early budding and late budding). Mature seeds were collected from main stems as well as subsequent branches for determination of germination, dormancy and seed viability.

MATERIAL AND METHODS

Fleabane seed collection and sowing Mature flaxleaf fleabane seeds were collected in summer 2013/14 near Wagga Wagga Agricultural Institute (WWAI), New South Wales, Australia. Plastic pots (20 cm in diameter and 18 cm in depth) were filled with field soil. The pots were irrigated to field capacity and two hundred mature seeds of fleabane were sown in the pots on 10 June 2014 in a glasshouse. After sowing, seeds were slightly harrowed to mix with the surface soil. The pots were then covered with a piece of tissue towel to maintain moist conditions for four days to encourage emergence. After the emergence, the pots were maintained with automatic irrigation on daily basis. Seedlings were manually thinned to two plants per pot at rosette stage.

Herbicide applications Herbicides were applied at reduced rates in an attempt not to kill the plants, with glyphosate applied at 360 g a.i ha^{-1} and paraquat at 100 g a.i ha^{-1}. The two herbicides were applied at two different growth stages of fleabane plants: at early budding (small visible buds just formed on the main stem) and late budding (large buds just about to open or slightly opened on the main stem) on 17 September and 11 October 2014, respectively. For both growth stages, the herbicides were applied 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. Pots without herbicide applications were included as controls.

Seed collection after herbicide treatments After the herbicide applications, mature seeds from the treated fleabane plants were collected between November 2014 and February 2015. Two sources of seeds were collected, seeds from the main stem (young buds directly exposed to herbicide application) and seeds from healthy branches (indirect effect from translocated herbicides). At the time of herbicide applications, the branches had not formed the buds yet. After the seed collections from the main stems and branches, the mature plants were then cut at the base (10 cm above the soil surface), on 20 January 2015 for the early budding (EB) treatments, and on 2 February 2015 for the late budding (LB) treatments, to assess residual herbicidal impacts on seeds produced on re-grown stems. The seeds produced from the re-grown stems were collected in March and April 2015, respectively. The collected seeds were tested for viability by both germination and tetrazolium tests.

Germination and viability tests For germination bioassay, one hundred of seeds from each treatment were placed onto 9 cm Petri dishes lined with one layer of filter paper (No. 1 Whatman). Five mL of distilled water was delivered to each Petri dish. Each Petri dish with its cover was sealed with a piece of Parafilm to reduce evaporation and was placed in growth incubators at 15°C/25°C on a 12 hour day/night cycle. After seven days of incubation, the germinated seeds were counted based on the emergence of cotyledons. Seeds that did not germinate were further assessed for viability by a tetrazolium test (Stanton *et al.* 2012). The tetrazolium [2,3-5-triphenyltetrazolium chloride (TTC) solution was added into a new Petri dish and 20 non-germinated seeds were left in contact with the solution for two days at room temperature. After this time, seeds which turned red in colour were classed as viable but dormant. The embryos that did not stain red were considered unviable. Total viable seeds include the germinated seeds and the 'viable but dormant' seeds.

Design and statistical analysis The experiments were designed as a randomised complete block with three replications. ANOVA was conducted using Genstat.

RESULTS AND DISCUSSION

Effects of herbicides on seed viability and dormancy of the maternal fleabane plants Data analysis confirmed significant ($P < 0.001$) differences in seed viability of fleabane in response to herbicides applied at the EB and the LB stages. Application of glyphosate at 360 g a.i ha^{-1} at the EB stage completely stopped the seedset on the main stem, while paraquat at 100 g a.i ha^{-1} did not affect fleabane seed production and viability on the main stem (Table 1). Glyphosate application at the EB stage desiccated the premature buds.

Glyphosate significantly affected the germination and dormancy of seeds collected from the earliest branches on the 10 December 2014, although the total seed viability was not affected as compared to the untreated control. The germination and dormancy was 9.3% and 69.8% for the glyphosate treatment, compared to 6.1% and 77.7% for the untreated control, respectively. The impact of glyphosate on seed germination and dormancy decreased over time when fleabane seeds were collected from later branches on or after 24 December 2014.

Table 1. Impact of herbicides on seed production and dormancy in *Conyza bonariensis*.

Treatment	Date of collection	Seed source	Germinable	Viable but dormant	Total viable
EARLY budding					
Glyphoste	20/11/14	Main stem	NA*	NA	NA
Glyphoste	10/12/14	Branch	9.3	69.8	79.1
Glyphoste	24/12/14	Branch	73.0	20.2	93.2
Glyphoste	2/01/15	Branch	73.3	17.0	90.3
Glyphoste	20/01/15	Branch	78.3	14.2	92.5
Paraquat	20/11/14	Main stem	61.0	23.7	84.7
Paraquat	2/01/15	Branch	49.0	14.1	63.1
Paraquat	20/01/15	Branch	65.0	9.3	74.3
Control	20/11/14	Main stem	66.7	8.6	75.3
Control	10/12/14	Branch	77.7	6.1	83.7
Control	24/12/14	Branch	73.3	6.7	80.0
Control	2/01/15	Branch	82.3	3.6	85.9
Control	20/01/15	Branch	80.7	3.7	84.4
$LSD_{0.05}$			13.5	5.6	15.5
LATE budding					
Glyphosate	19/11/14	Main stem	34.0	26.8	60.8
Glyphosate	23/01/15	Branch	30.0	18.6	48.6
Glyphosate	2/02/15	Branch	68.0	10.7	78.7
Paraquat	19/11/14	Main stem	28.0	30.7	58.7
Paraquat	23/01/15	Branch	56.3	19.7	76.0
Paraquat	2/02/15	Branch	45.3	10.7	56.1
Control	19/11/14	Main stem	77.7	4.6	82.2
Control	23/01/15	Branch	80.7	3.7	84.4
Control	2/02/15	Branch	76.3	5.2	81.5
$LSD_{0.05}$			14.4	6.7	13.2

*Glyphosate application at the early budding stage completely killed the premature buds.

The contact herbicide paraquat at 100 g a.i. ha^{-1} applied at the EB stage had little desiccation effect on the premature buds and it only desiccated some leaves. The plant recovered completely and the young buds continued to progress and set seeds, resulting in no differences in seed viability compared to the untreated control. However, paraquat-treated seeds on the main stem were more dormant (23.7%) than the untreated control (8.6%). The impact of paraquat on dormancy also declined as the seed collection time progressed from 20 November 2014 to January 2015. When collected on 20 January 2015, its impact on dormancy dropped to 9.3%., which is not significantly different to the untreated control (3.7%) for the same period.

Herbicide applications at LB stage did not stop the growth of fleabane plants which continued to develop, flower and set seeds. However, the LB application of glyphosate and paraquat also had significant effects on seed viability and dormancy. Both herbicides reduced seed viability on the main stem by 26–29% but increased the dormancy by 5–6 times when compared with the untreated control (Table 1). The herbicides also induced the dormancy of seeds collected from branches. However, the herbicidal impact on dormancy decreased as the seed collection progressed. These results suggest that fleabane seed dormancy could be induced by herbicides, depending on the herbicide and the application timing. This finding is in contrast with

the previous report that seeds did not possess innate dormancy (Wu *et al.* 2007). The discrepancy could be partly due to the seed used by Wu *et al.* (2007) was not from fleabane plants after herbicide application. In addition, the seed has been stored for a few months prior to the experiments. Any innate dormancy might have been lost during storage as shown in the current study.

Dormancy release after ripening The induced dormancy due to herbicides underwent a steady decline during storage (Figure 1). The dormant seeds (%) decreased significantly from the initial 26.8% in the glyphosate treatment to 2.3% after seven months of storage under room temperature. The significant dormancy release in the paraquat treatment also ranged from 30.7% to 0.3%, while there was no difference in the dormancy status in the control treatments, with an average of 3.6%.

Carry-over effects of herbicides on seed viability and dormancy of the re-grown fleabane plants after cutting Glyphosate and paraquat did not have any significant effects at either EB or LB stage on viable-but-dormant seeds collected from re-grown plants after cutting, when compared with the untreated control (Table 2). However, glyphosate applied at the EB stage and paraquat applied at the LB stage significantly reduced the germinable seeds and the total viable seeds.

The impact of both herbicides on seed viability is in general accordance with previous research. Carrithers *et al.* (2004) found that picloram applied at bud stage reduced seed production of yellow starthistle (*Centaurea solstitialis* L.) by 42 to 86% and viability by 80 to 99%. Application of picloram, which caused both bud abortion and lower seed germination, was more effective at the bud stage than the flowering stage in reducing viable seed. Jha and Norsworthy (2012) found that a range of herbicides applied at the first visible sign of inflorescence reduced seed production of glyphosate resistant Palmer amaranth (*Amaranthus palmeri*) by 75 to 95% depending on the biotypes. The herbicides also reduced viability, with seeds produced by treated plants being 45 to 61% viable, compared with 97% seed viability in non-treated plants. Keenan *et al.* (2014) also reported that a range of herbicides can reduce seed viability of fleabane by up to 100%, depending on the application timing and the herbicide.

These studies demonstrate that herbicides could potentially reduce seed production and viability of many agricultural weeds, thereby reducing seedbank replenishment and subsequent impact in the following season. However, on the other hand, herbicide applications could also induce seed dormancy, which could prolong the seed persistence in the field, adding another layer of complexity to seedbank management. The herbicide-induced dormancy reported in the present study could contribute to the altered seed dormancy found in *H. murinum*, *B. diandrus* and *L. rigidum* in intensive cropping systems which relied heavily on herbicide weed control (Fleet and Gill 2012, Kleemann and Gurjeet 2013, Owen *et al.* 2015).

More research is needed to determine if herbicides applied at seed maturation will have broad effects on seed dormancy in other weeds. Further studies on the physiological and molecular responses to herbicidal applications might unravel the mechanisms for this dormancy induction.

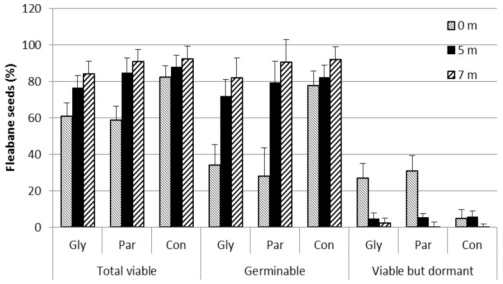

Figure 1. Reduction in fleabane dormancy after ripening.

Table 2. Herbicide carry-over impacts on viability and dormancy of seeds collected on 13 April 2015 from the re-grown fleabane plants after cutting.

Herbicide	Germinable seeds (%)	Viable but dormant seeds (%)	Total viable seeds (%)
EARLY budding			
Glyphosate	25.3a	8.8a	34.1a
Paraquat	95.0b	1.3a	96.3b
Control	69.3c	11.2a	80.5b
LATE budding			
Glyphosate	77.3c	8.9a	86.2b
Paraquat	54.7d	14.4a	69.0c
Control	74.0c	11.6a	85.6b

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