Glyphosate resistance and herbicide effects on cypsela (seed head) production in sowthistle

Abul Hashem, Catherine Borger and Mohammad Amjad, Department of Primary Industries and Regional Development, Northam

Key messages

- Two populations of sowthistle collected from the roadside of the Western Australian wheatbelt were found resistant to glyphosate.
- A single application of herbicide or tank mix of herbicides may not kill sowthistle plants but significantly reduces cypsela (seed head) production
- Double knockdowns should be employed as part of an integrated weed management strategy to effectively control sowthistle in Western Australia.

Aims

In recent years, common sowthistle (*Sonchus oleraceus*) infestation has increased along the roadsides of Western Australia (WA) (Hashem et al 2016, Borger et al 2017). Common sowthistle, also known as milk thistle, is widespread across the grain-growing regions of Queensland and northern New South Wales (Widderick and Walker 2009). The increase in sowthistle is thought to be related to changes in Australian farming systems including a trend for growers to reduce the number of tillage operations and rely more on herbicides for weed control. The technique of 'Double-Knock'; glyphosate followed by paraquat is effective on sowthistle at various stages. However, sowthistle may survive and produce seed when treated with only a single application of herbicide(s). The ability of sowthistle to emerge and grow during both summer and winter is of great concern for winter grain crops in WA. In the eastern states of Australia, 23 populations of sowthistle have developed resistance to glyphosate (Preston 2018). Given the recent widespread occurrence of sowthistle along the roadside across the grain growing areas of WA, it is necessary to assess sowthistle populations for resistance to glyphosate and the potential to reduce seed production should the weed survive herbicide application.

Methods

Herbicide resistance test (Experiment 1)

Mature seed of 15 populations of sowthistle were collected from WA roadsides during the 2014/2015 summer weed survey (Hashem et al 2016). Seeds of all sowthistle populations were stored in laboratory conditions (23^{O} C) at the Department of Primary Industries and Regional Development (DPIRD), Merredin. A glasshouse resistance test with eight herbicide treatments (Table 1) and an untreated control was established at Merredin in October 2015 in a completely randomised design with three replications. The bottom half of each pot (1.8 L) was filled with potting mix and the top half filled with sand. The pots were then soaked with water two hours before sowing the seed. Sowthistle seed was spread on the surface of the pot and then covered with a thin (5 mm) layer of sand. Pots were watered daily to avoid any water stress and maintained under glasshouse conditions at 23/15^{o} C (day/night cycle). After emergence of the seedlings, the plants were thinned to 12-15 plants per pot. At the 3-4 leaf stage, all treatments (Table 1) were applied using a closed-door overhead compressed-air belt-driven glasshouse sprayer calibrated to deliver 96 L ha^{-1} output at 200 kPa pressure (with flat fan nozzles). An untreated control was maintained for each population. Plants were returned to the glasshouse with no water applied for 24 hours after spraying. Plant survival was assessed one week after application of paraquat + diquat, two weeks after 2,4-D, and four weeks after metsulfuron and glyphosate. Plant survival in the treated pots was expressed as a percentage of untreated control. A plant was assessed as surviving if it was not affected by herbicide or affected but still actively regrowing with visible green shoots and new leaves. A plant was assessed dead if it had visibly senesced with no regrowth, or if part of the shoot base was green but the central growing point was completely dead.

Glyphosate dose response test (Experiment 2)

A dose response experiment was conducted under glasshouse conditions at Northam in 2016 using four populations (two susceptible and two resistant) selected from Experiment 1 to generate a dose response curve to determine the resistance level and the LD50. Each population was treated with five rates of glyphosate 0, 500, 1000, 2000 and 400 g ai/ha) to generate the dose response curve, replicated three times in a completely randomised design. The experiment was conducted following the methodology detailed in Experiment 1.

Herbicide effects on the growth suppression and seed production potential of sowthistle (Experiment 3):

Figure 1 Sowthistle plants wrapped with colourless bridal fabric to collect all the seed released after maturity of cypsela at Northam in 2016/2017.

Mature seed of one sowthistle population growing in a fallow paddock was collected from a paddock near Geraldton Airport in mid-July 2015. Seed was stored under glasshouse conditions at 23/15^{O}C (day/night cycle) until use in mid-August 2015. Sowthistle seedlings were raised and herbicide treatments applied following the methodology described in Experiment 1. Seedlings were sprayed (9 September 2015) with the herbicides and rates in Table 3. Ten days after application, half of the pots from each treatment with surviving plants were transferred outdoors and the remainder maintained in the glasshouse. The available photosynthetically active radiation (PAR) inside the glasshouse ranged from 600 to 1000 µmol/m^2/s. Approximately 600 µmol/m^2/s of PAR is considered enough for plant growth if irradiance is available for at least 12 hours per day (Dietzer et al 1994). The PAR available for the outdoor plants varied from 1200 to 1800 µmol/m^2/s. The suppression in the growth of sowthistle plants was assessed visually three weeks after spraying and expressed as percent of the untreated control. At flowering, all plants were wrapped with bridal fabric (Figure 1) to enable all seed to be collected as plants matured. Total number of cypsela (seed head) produced per plant from 11 October 2015 to 4 January 2016 were counted and expressed as a percentage of the untreated control.

Design and analysis: Data were subjected to ANOVA. LD_{50} ratio was estimated by probit analysis in experiment 2 and means were separated by Lsd in Experiment 2 and 3 (GENSTAT 18th Edition).

Results

Glyphosate resistance in sowthistle (Experiment 1):

Out of the fifteen populations of sowthistle tested, 4 populations had plants that had survived the highest label rate of glyphosate (Table 1). When treated with metsulfuron, plants in four populations of sowthistle survived at highest label rates. These results suggest that some populations of sowthistle are developing resistance to glyphosate and metsulfuron. It is necessary to perform a dose response test to confirm the resistance to glyphosate in these populations. No sowthistle plants of any population survivied 2,4-D or paraquat + diquat.

Table 1 Survival of sowthistle populations collected from the roadside of the Western Australian Wheatbelt during the 2014/2015 summer and treated with various herbicides under glasshouse conditions at Merredin in Experiment 1.

Herbicide	Herbicide rate gi/ha	Number of resistant populations
Glyphosate	1080	4
Paraquat + diquat	500	0
2,4-D E (LVE)	600	0
Metsulfuron	21	4

Estimation of resistance level in sowthisle populations (Experiment 2): A dose response test showed that all the sowthistle plants of population 71 had died when treated with glyphosate at 500g/ha but 17% of plants from population 79 survived this rate (Figure 2). However, all the plants of population 79 died when treated with glyphosate at 1000g/ha. In population 29, all plants died when treated with glyphosate at 500g/ha but 6% of plants from population

80 survived at this rate (Figure 2). All the plants from population 80 died when treated with glyphosate at 1000g/ha. These results suggest that resistance to glyphosate has evolved in populations 79 and 80. Estimation of LD_{50} ratio by probit analysis showed that population 79 was 2.75-fold more resistant than population 71 while population 80 was 2.68-fold more resistant than population 29. (Figure 2). Since the LD_{50} ratios were above 2, these results confirm that sowthistle population 79 and 80 have evolved a low level of resistance to glyphosate.

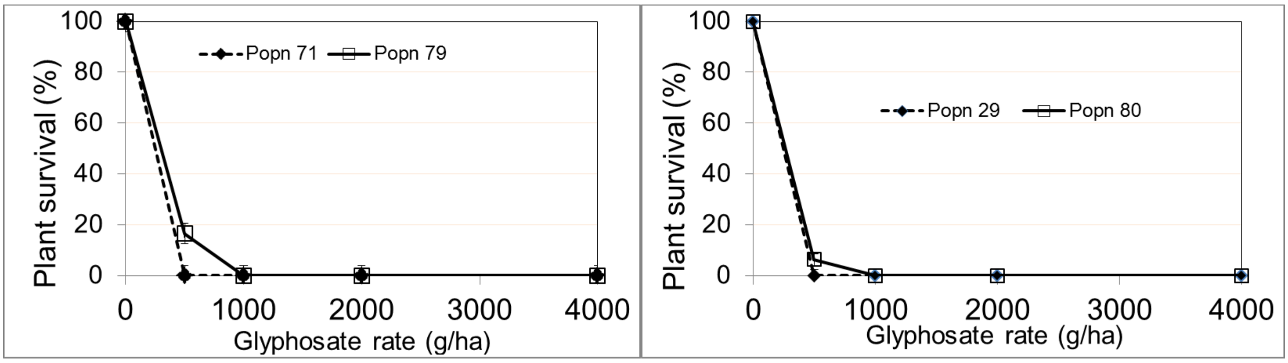

Figure 2 Response of four sowthistle populations collected from roadsides of Western Australia during 2014/2015 summer to a range of glyphosate doses under glasshouse conditions at Northam. Population 29 and 71 were susceptible while population 79 and 80 were resistant.

Herbicide effects on the survival, growth and cypsela production of sowthistle (Experiment 3): Plant survival was significantly lower (4% to 14%) in the pots treated with herbicides compared to untreated pots. Where plants survived, growth was suppressed (53% to 91%) (Table 2). The level of plant survival was higher with bromoxynil + pyrasulfotole than other herbicides. Suppression levels by 2,4-D or paraquat + diquat were greater than glyphosate or bromoxynil + pyrasulfotole (Table 2).

Table 2 –Effect of herbicides on plant survival and growth suppression measured three weeks after spraying a population of sowthistle collected from Geraldton and tested under glasshouse conditions at Northam in Experiment 3.

Herbicide and rate g ai/ha	Plant survival (%)	Growth suppression (%)
Glyphosate 350	4	53
Bromoxynil 147 + pyrasulfotole 26	14	66
Paraquat 500 + amitrole 2.5	4	74
2,4-D 625	4	91
No herbicide	100	0
P value	<.001	<.001
Lsd (5%)	10.70	21.92

When half of the pots were transferred outdoors and the remainder maintained inside the glasshouse, cypsela (seed head) production was reduced by 47% to 52% under glasshouse conditions and 26% to 74% under outdoor conditions compared to the untreated control (Table 3). While the reduction in the cypsela production among the herbicide treatments did not vary significantly (p-value 0.1) under glasshouse conditions, a greater reduction in the number of cypsela was recorded by 2,4-D compared to bromoxynil + pyrasulfotole when maintained outdoors (Table 3). However, the average number of cypsela produced under glasshouse (36/plant) was 2.5 times lower than under outdoor conditions (92/plant) (Table 3).

Table 3 –Effect of herbicides on the cypsela production of a sowthistle population collected from the Geraldton area and tested under glasshouse and outdoor conditions as compared to the untreated control at Northam in Experiment 3.

Herbicide rate g ai/ha	Reduction in cypsela (%)*	
	Glasshouse	Outdoor
Glyphosate 350	47	47
Bromoxynil 147 + pyrasulfotole 26	57	26
Paraquat 500 + amitrole 2.5	50	51
2,4-D 625	52	74
No herbicide	-	-
P-value	NS	0.014
SE	22.28	25.65

*In the untreated control cypsela production was 36/plant under glasshouse and 92/plant under outdoor conditions.

Conclusions

Two populations of sowthistle collected from roadsides of the WA wheatbelt have developed resistance to glyphosate. The resistance level in these populations is still low but this may increase further if roadside weed managers or growers continue to use glyphosate every year for sowthistle control. If this management strategy continues, managers and growers may fail to control this weed with label rates of glyphosate once a high level of resistance has developed. While the actual level of glyphosate resistance is still low in WA, experience with sowthistle populations in New South Wales with a similar resistance level shows that even this low level of glyphosate resistance is enough to result in increased weed numbers in paddocks being treated with glyphosate only. To manage sowthistle and delay further development of glyphosate resistance in this and other emerging weed species, growers should apply the principles of integrated weed management (IWM). Since a small proportion of sowthistle plants are likely to survive a single application of herbicides, double knock applications should be employed as IWM to effectively manage this weed in WA. Other examples of a successful IWM program may include improving spray application techniques and using other non-chemical weed control options along roadsides. Although herbicides can significantly reduce the cypsela production of the surviving plants, the viability of seed produced by affected plants needs to be assessed. For more information visit: http://www.glyphosateresistance.org.au/

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Key words

Sowthistle, glyphosate resistance, double knockdown, growth suppression, cypsela production

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