Extent of herbicide resistance in three broadleaf weed species in southern New South Wales

John C. Broster[1], Eric A. Koetz[2], Adam J. Shephard[2] and Hanwen Wu[2]
Graham Centre for Agricultural Innovation
(NSW Department of Primary Industries and Charles Sturt University)
[1] Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia
[2] Wagga Wagga Agricultural Institute, PMB, Wagga Wagga, NSW 2650, Australia
(JBroster@csu.edu.au)

Summary Across the cropping region of southern New South Wales the majority of paddocks contain populations of grass weeds resistant to herbicides. With broadleaf weeds also treated with herbicides in many of these paddocks and the high levels of resistance found in Western Australian wild radish populations, two field surveys of crops in southern New South Wales in 2011 and 2012 collected samples of wild radish, Indian hedge mustard and sow thistle. In 2011, 35 paddocks were visited, resulting in 11 wild radish samples being collected while 143 paddocks were visited in 2012 from which 19 wild radish, 20 Indian hedge mustard and 79 sow thistle samples were collected. The samples were screened between June and August the year after collection to a number of different herbicides. In both surveys samples were only found to be resistant to Group B herbicides with one wild radish sample from the 2011 survey resistant to chlosulfuron and imazamox + imazapyr and another developing resistance to imazamox + imazapyr. From the 2012 survey samples 35% of the sow thistle samples (28/79) were resistant to metsulfuron-methyl and 15% of wild radish samples (3/19) were resistant to chlorsulfuron, while no Indian hedge mustard samples were resistant to chlorsulfuron. Other herbicides screened to which no resistance was found included atrazine, terbuthylazine, diflufenican, 2,4-D amine or glyphosate. The absence of resistance to any herbicides other than the Group B herbicides is good news for farmers in southern New South Wales as they still have several options for controlling these broadleaf weeds.

Keywords Herbicide resistance, wild radish.

INTRODUCTION

Herbicide resistant weeds are considered a major problem in the cropping regions of Australia. The first case of herbicide resistance in Australia was reported in 1980, a population of annual ryegrass (*Lolium rigidum* Gaud.) resistant to diclofop-methyl (Heap and Knight 1982). Subsequently resistance has been reported to wild oats (*Avena fatua* L.) also to diclofop-methyl (Piper 1990) and in two species of barley grass (*Hordeum glaucum* Steud. and *H. leporinum* Link) to paraquat in 1982 and 1987 respectively (Powles 1986 and Tucker and Powles 1991). In dicot weeds the first cases of resistance in Australia were reported in 1995 with biotypes of both sow thistle (*Sonchus oleraceus* L.) and Indian hedge mustard (*Sisymbrium orientale* Torn.) found to be resistant to chlorsulfuron (Boutsalis and Powles 1995). Subsequently populations of wild radish (*Raphanus raphanistrum* L.) in Western Australia were found to be resistant to chlorsulfuron and metosulam (Hashem *et al.* 2001 and Walsh *et al.* 2001).

Random surveys across the Western Australian wheat belt have reported high levels of herbicide resistance in wild radish populations. Walsh *et al*. (2001) reported 21% of populations collected in a 1999 random survey were resistant to chlorsulfuron. A subsequent survey in 2003 found the proportion of populations that contained chlorsulfuron resistant wild radish had increased 54%. Additionally, 60% of the surveyed populations contained plants resistant to 2,4-D amine, 40% to diflufenican and 15% to atrazine with only 17% of populations still susceptible to all four tested herbicides (Walsh *et al.* 2007).

Wild radish was rated by Western Australian farmers as their second most difficult weed to control, behind only annual ryegrass (Jones *et al.* 2000), compared to fourth in southern New South Wales, Victoria and South Australia. Compared to Western Australia, in southern New South Wales wild radish is less common. A survey in 1993 found wild radish in only 3.5% of paddocks (Lemerle *et al.* 1996), a similar level (3.6%) was also reported in a more recent survey conducted in 2010 (Broster *et al.* 2012).

Prior to the research described here, no surveys to determine the resistance status of wild radish, sow thistle or Indian hedge mustard had been conducted in southern New South Wales.

MATERIALS AND METHODS

Survey Cropping paddocks in southern New South Wales were surveyed in November 2011 and November and December 2012 prior to the commencement

of harvest. Paddocks were randomly selected at 10 km intervals, alternating left and right hand side of the survey transects where possible. Additionally, in 2011 every second pulse crop was also visited. This resulted in 35 paddocks being visited in 2011 and 143 in 2012 for a total of 176 of which 72 had no broadleaf weeds present. The majority of paddocks were wheat (132), with 17 lupin, eight oats, six barley, five field pea, three canola, two faba bean and two triticale crops also visited. The location of all sites was recorded using a GPS unit.

The paddocks were surveyed by two people walking across them for a ten to fifteen minute period. In 2011 only wild radish was collected (11 samples) while in 2012, wild radish (19 samples), sow thistle (79 samples) and Indian hedge mustard (20 samples) were collected. In the 2012 survey the majority of paddocks contained only one of these species (68), however 28 of the paddocks contained two and two paddocks contained all three species.

Resistance screening The samples from the 2011 survey were sown in July 2012 with 25 seeds placed in each pot. For all samples three replicates were sown. Two weeks after sowing all samples were counted. Pots were kept in a temperature controlled glasshouse (10°C minimum, 25°C maximum) and watered and fertilised as required. All pots were filled with a soil mix (50:50 loam:river wash sand). Due to low seed numbers not all samples were screened to all herbicides.

The wild radish samples collected in 2011 were screened with six post-emergent herbicides from Groups B (chlorsulfuron and imazamox + imazapyr), C (atrazine), F (diflufenican), I (2.4-D amine) and M (glyphosate). The 2012 wild radish and Indian hedge mustard samples were screened to the same herbicides except for imazamox + imazapyr, while the sow thistle samples were screened to metsulfuron, terbuthylazine, 2,4-D amine and glyphosate. All post emergent herbicides were applied at the rate and plant growth stage recommended on the herbicide label. Terbuthylazine was applied pre-emergent, was incorporated; the seeds were sown and then incorporated again (Table 1).

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. Adjuvants were added to herbicides as recommended by the label. A standard susceptible biotype and a known resistant biotype, where available, were included with each cohort of samples, with each herbicide tested in a completely randomized design.

Table 1. Herbicides and rates used for resistance screening (adjuvants added as recommended by the label).

Herbicide	Group	Rate (g a.i. ha^{-1})
Chlorsulfuron	B (SU)	15
Metsulfuron	B (SU)	3
Imazamox + imazapyr	B (imi)	16.5 + 7.5
Atrazine	C	1000
Terbuthylazine	C	1050
Diflufenican	F	100
2,4-D amine	I	875
Glyphosate	M	648

Herbicide evaluation All samples were assessed 28 days after treatment. For the post emergent herbicides seedlings were counted before and after treatment to enable survival percentages to be calculated. For the pre-emergent herbicide, terbuthylazine, the samples were rated visually from 0 (no germination) to 10 (no visual difference from susceptible control). Results were analysed by ANOVA and the standard error for each herbicide determined. As the standard error for none of the herbicides screened exceeded 1.0 for the pre-emergent or 10% for the post emergent herbicides, standard thresholds were used as this ensured samples classified as developing resistant or resistant were significantly different from the susceptible control. Samples were classified as resistant if the mean survival percentage was greater than 20% for post-emergent herbicides while samples with survival percentages of between 10 and 19% were classified as developing resistance. A visual score of greater than 2 for pre-emergent herbicide was classed as resistant and a visual score of between 1 and 2 as developing resistance.

RESULTS

Wild radish Over the two surveys, two samples were classed as resistant to chlorsulfuron and two as developing resistance. Of the ten samples from the 2011 survey screened to chlorsulfuron and imazamox + imazapyr, one sample was resistant to both herbicides. Another sample was classed as developing resistance to imazamox + imazapyr but susceptible to chlorsulfuron, although some plants from this sample survived the chlorsulfuron application the level of survival was below the threshold to be classed as developing resistant. One of the 19 samples from the 2012 survey was classed as resistant to chlorsulfuron and another two as developing resistance.

None of the wild radish samples from either the 2011 or 2012 surveys were found to be resistant to the herbicides atrazine, diflufenican, 2,4-D amine or glyphosate.

In the 2011 survey one sample had a low level of survival to chlorsulfuron and imazamox + imazapyr and another to imazamox + imazapyr only, while in the 2012 survey one sample had some survival to chlosulfuron, all without reaching the developing resistance threshold. In the 2011 survey one sample had a single plant survive the 2,4-D amine application and two others had single surviving plants after glyphosate application while no samples from the 2012 survey had any surviving plants to a herbicide other than chlosulfuron.

Table 2. Wild radish resistance levels (2011 and 2012 surveys combined) for the screened herbicides (R – resistant; DR – developing resistance; TR – total resistant = resistant and developing resistance combined).

Herbicide	Tested (no.)	R (no.)	DR (no.)	**TR (%)**
Chlorsulfuron	29	2	2	**14**
Imazamox + imazapyr	10	1	1	**20**
Atrazine	24	0	0	**0**
Diflufenican	27	0	0	**0**
2,4-D amine	28	0	0	**0**
Glyphosate	24	0	0	**0**

Sow thistle Over 20% of the samples were classed as resistant to metsulfuron with an additional 14% developing resistance (Table 3). Twenty one of the 51 samples classified as susceptible also had some plants survive the chlosulfuron application but at levels below the threshold for developing resistance.

Table 3. Sow thistle resistance levels for the screened herbicides (R – resistant; DR – developing resistance; TR – total resistant = resistant and developing resistance combined).

Herbicide	Tested (no.)	R (no.)	DR (no.)	**TR (%)**
Metsulfuron	79	17	11	**35**
Terbuthylazine	61	0	0	**0**
2,4-D amine	78	0	0	**0**
Glyphosate	75	0	0	**0**

All sow thistle samples were susceptible to the herbicides terbuthylazine, 2,4-D amine or glyphosate. Seven of the samples had a single surviving plant after the glyphosate application but no surviving plants were found to the other herbicides.

Indian hedge mustard All 20 Indian hedge mustard samples were classed as susceptible to all of the herbicides screened. However, surviving plants were found to both glyphosate (8 samples) and chlorsulfuron (1 sample).

DISCUSSION

Thirteen percent of the wild radish populations screened in the two surveys were resistant to a Group B herbicide (chlorsulfuron and/or imazamox + imazapyr) significantly lower than in Western Australia where 54% were found to be resistant to chlorsulfuron (Walsh *et al.* 2007). Additionally, unlike Western Australia, no populations were found to be resistant to the Group C, F or I herbicides tested.

Wild radish samples screened by the commercial herbicide resistance testing service at Charles Sturt University (CSU) have shown similar trends to the results of these surveys. Between 2001 and 2013, 31% of wild radish samples from New South Wales (26 tested) were resistant to Group B herbicides, compared with 60% of samples from Western Australia (163). As was found in the surveys, no New South Wales samples were resistant to other herbicide groups compared to the Western Australian samples with of 2%, 19% and 15% of samples resistant to Group C, F or I herbicides respectively (J. Broster unpublished data).

This difference is not found in annual ryegrass where surveys have reported minimal differences between New South Wales and Western Australia (Owen *et al.* 2007, Broster *et al.* 2011 and Broster *et al.* 2013). Annual ryegrass is considered the major weed in both regions and the lower incidence of wild radish in New South Wales (Lemerle *et al.* 1996 and Broster *et al.* 2012) compared with Western Australia may be a major factor in the slower development of herbicide resistance due to lower herbicide use for its control and therefore decreased selection pressure. The mixed farming systems in southern New South Wales, often with a pasture phase featuring lucerne, could also have influenced both the presence of wild radish and the frequency of herbicide applications for its control.

The higher level of resistance in sow thistle compared with the wild radish may be related to the higher incidence of this weed in the surveyed area. The 2012 survey collected 79 sow thistle samples compared with 19 wild radish samples. Both Lemerle *et al*. (1996) and Broster *et al*. (2012) reported a greater proportion

of paddocks contained sow thistle (10.5% and 23% respectively) than wild radish (3.5% and 3.6% respectively). The findings of this survey are similar to that of a semi-targeted survey conducted around Griffith (NSW) in 2012 that found 19% of sow thistle populations (31 tested) resistant or developing resistance to metsulfuron and one population developing resistance to imazapic + imazapyr (B. Haskins pers. comm.).

Although no Indian hedge mustard populations were found to be resistant, resistant populations from New South Wales have been recorded in populations tested by the CSU testing service (J. Broster unpublished data).

For all three species, and many of the herbicides, in addition to the populations classed as resistant or developing resistant other populations contained plants surviving at the time of assessment. While these may be resistant plants it could be that they have avoided the herbicide application by various methods (e.g. late germination, sheltered by other plants) and the survival percentage for the sample was not significantly different from a known susceptible population.

While these surveys show that the extent of herbicide resistance in three broadleaf weed species is lower than in other regions of Australia, especially Western Australia, it is still significant. While surveys late in the season only found wild radish in a small proportion of paddocks, agronomists rated it as the 11th most common weed species, 6th hardest weed to control and the 2nd most important weed behind annual ryegrass in terms of being a serious problem in the future (Lemerle *et al.* 1996). Herbicide resistance will only make this and associated weed species more of a problem in the future.

ACKNOWLEDGMENTS

This work was funded by a Grains Research and Development Corporation Grant (CSU00113). Thanks to Rylie Cherrey for assistance in the resistance screening.

REFERENCES

Boutsalis, P. and Powles, S.B. (1995). Resistance of dicot weeds to acetolactate synthase (ALS)-inhibiting herbicides in Australia. *Weed Research* 35, 149-55.

Broster, J.C., Koetz, E.A. and Wu, H. (2011). Herbicide resistance levels in annual ryegrass (*Lolium rigidum* Gaud.) in southern New South Wales. *Plant Protection Quarterly* 26, 22-8.

Broster, J.C., Koetz, E.A. and Wu, H. (2012). Weed species present in cereal crops in southern New South Wales. Proceedings of 18th Australasian Weeds Conference, ed. V. Eldershaw, pp. 241-4. (Weed Society of Victoria, Melbourne).

Broster, J.C., Koetz, E.A. and Wu, H. (2013). Herbicide resistance levels in annual ryegrass (*Lolium rigidum* Gaud.) and wild oats (*Avena* spp.) in south-western New South Wales. *Plant Protection Quarterly* 28, 126-32.

Hashem, A., Bowran, D., Piper, T. and Dhammu, H.S. (2001). Resistance of Wild Radish (*Raphanus raphanistrum*) to Acetolactate Synthase inhibiting herbicides in the Western Australian wheat belt. *Weed Technology* 15, 68-74.

Heap, J.W. and Knight, R. (1982). A population of ryegrass tolerant to the herbicide diclofop-methyl. *Journal of the Australian Institute of Agricultural Science* 48, 156-7.

Jones, R., Alemseged, Y., Medd, R. and Vere, D. (2000). 'The distribution, density and economic impact of weeds in Australian annual winter cropping systems.' (CRC for Weed Management Systems, Adelaide).

Lemerle, D., Tang, H., Murray, G. and Morris, S. (1996). Survey of weeds and diseases in cereal crops in the southern wheat belt of New South Wales. *Australian Journal of Experimental Agriculture* 36, 545-54.

Owen, M.J., Walsh, M.J., Llewellyn, R.S. and Powles, S.B. (2007). Widespread occurrence of multiple herbicide resistance in Western Australian annual ryegrass (*Lolium rigidum*) populations. *Australian Journal of Agricultural Research* 58, 711-8.

Piper, T.J. (1990). Field trials on diclofop-methyl tolerant wild oats (*Avena fatua*). Proceedings of 9th Australian Weeds Conference. ed. J.W. Heap, pp. 211-5. (Crop Science Society of South Australia, Adelaide).

Powles, S.B. (1986). Appearance of a biotype of the weed, *Hordeum glaucum* Steud., resistant to the herbicide paraquat. *Weed Research* 26, 167-72.

Tucker, E.S. and Powles, S.B. (1991). A Biotype of Hare Barley (*Hordeum leporinum*) Resistant to Paraquat and Diquat. *Weed Science* 39, 159-62.

Walsh, M.J., Duane, R.D. and Powles, S.B. (2001). High frequency of chlorsulfuron-resistant wild radish (*Raphanus raphanistrum*) populations across the Western Australian wheatbelt. *Weed Technology* 15, 199-203.

Walsh, M.J., Owen, M.J., Powles, S.B. (2007). Frequency and distribution of herbicide resistance in *Raphanus raphanistrum* populations randomly collected across the Western Australian wheatbelt. *Weed Research* 47, 542-50.