Research Article

Received: 20 February 2014 Revised: 6 April 2014 Accepted article published: 25 April 2014 Published online in Wiley Online Library: 15 May 2014

(wileyonlinelibrary.com) DOI 10.1002/ps.3815

# Identification of the first glyphosate-resistant wild radish (*Raphanus raphanistrum* L.) populations

**Michael B Ashworth,[a] Michael J Walsh,[a] Ken C Flower[b] and Stephen B Powles[a*]**

## Abstract

**BACKGROUND: In Australia, glyphosate has been used routinely to control wild radish (*Raphanus raphanistrum* L.) for the past 40 years. This study focuses on two field-evolved glyphosate-resistant populations of wild radish collected from the grainbelt of Western Australia.**

**RESULTS: Two wild radish biotypes were confirmed to be glyphosate resistant by comparing R/S of two suspected populations. Based on R/S from dose–response curves, the R1 and R2 populations were 2.3 and 3.2 times more resistant to glyphosate respectively. Dose response on glyphosate-selected progeny (>1080 g ha$^{-1}$) demonstrated that the glyphosate resistance mechanism was heritable. When compared with the pooled mortality results of three known susceptible populations (S1, S2 and S3), the R1 and R2 subpopulations were 3.4-fold and 4.5-fold more resistant at the $LD_{50}$ level respectively. Both populations were found to have multiple resistance to the phytoene desaturase inhibitor; diflufenican, the synthetic auxin; 2,4-D and the ALS inhibitors; chlorsulfuron, sulfometuron-methyl, imazethapyr and metosulam.**

**CONCLUSIONS: This is the first report confirming glyphosate resistance evolution in wild radish and serves to re-emphasise the importance of diverse weed control strategies. Proactive and integrated measures for resistance management need to be developed to diversify control measures away from glyphosate and advance the use of non-herbicidal techniques.**


*Supporting information may be found in the online version of this article.*

**Keywords:** glyphosate; glyphosate resistance; wild radish; evolution; herbicide resistance

## 1 INTRODUCTION

The herbicide glyphosate [*N*-(phosphonomethyl) glycine] is unique in acting on the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). EPSPS inhibition limits aromatic amino acid biosynthesis,[1] inhibiting the biosynthesis of aromatic plant metabolites required for anthocyanins, lignin, growth promoters, growth inhibitors, phenolics and proteins.[2,3] Glyphosate is very effective in controlling a wide spectrum of annual and perennial weeds and has become an essential component in conservation farming systems worldwide as a substitute for tillage. In addition, it is widely used in a range of recreational and industrial areas. Reliance on glyphosate for weed control dramatically increased following the widespread adoption of transgenic glyphosate-resistant crops.[4] As a result of glyphosate overreliance, resistance has evolved in 25 weed species (12 dicots, 13 monocots) worldwide.[5] In Australian cropping systems, glyphosate resistance has evolved through preseeding and fallow glyphosate use, with resistance confirmed in annual ryegrass (*Lolium rigidum* L.),[6,7] flaxleaf fleabane (*Conyza bonariensis* L.),[8] liverseed grass (*Urochloa panicoides* L.), jungle rice (*Echinochloa colona* L.)[9] and windmill grass (*Chloris truncate* L.).[5]

Wild radish (*Raphanus raphanistrum* L.) is an important weed of agricultural regions, listed in the top 180 worst weeds worldwide. It is the most economically damaging dicotyledonous weed species of cropping systems in the Western Australian (WA) grainbelt,[10–12] aggressively competing for water, nutrients and light.[13–15] Wild radish is problematic in rainfed cropping systems because it germinates over a wide range of environmental conditions, has a flexible life cycle and is a prolific seed producer. Seeds are innately dormant, and this characteristic contributes to a persistent soil seed bank.[13,16] Continuous herbicide use on large genetically diverse populations[17] has resulted in the evolution of resistance to multiple herbicides, including inhibitors of acetolactate synthase

* *Correspondence to: Stephen B Powles, Australian Herbicide Resistance Initiative, School of Plant Biology, University of Western Australia, WA 6009, Australia. E-mail: stephen.powles@uwa.edu.au*

a *Australian Herbicide Resistance Initiative, School of Plant Biology, University of Western Australia, WA, Australia*

b *School of Plant Biology and Institute of Agriculture, University of Western Australia, WA, Australia*

(ALS),[18–20] phytoene desaturase (PDS),[19] photosynthetic electron transport (PSII)[19,21] and synthetic auxin herbicides.[19,20]

In Australia there is a high reliance on glyphosate, raising concerns about glyphosate resistance evolution in wild radish populations. Here, the authors report the first documented case of glyphosate-resistant wild radish by characterising the level of resistance and evaluating multiple resistance in two field-collected wild radish populations from the grainbelt of Western Australia.

## 2 MATERIALS AND METHODS

### 2.1 Collection of suspected glyphosate-resistant wild radish populations

In 2010, two field sites were identified where wild radish was not controlled with the recommended glyphosate rates (>540 g ha$^{-1}$). One population was collected near Mingenew (latitude −29.19 S, longitude 115.44 E) (referred to hereinafter as R1) while the other site was 200 km south near Carnamah (latitude 28.68 S, longitude 115.89 E) (referred to hereinafter as R2) (Table 1) in the northern grainbelt of Western Australia. At each site, mature seed-containing pod siliques were collected from a minimum of 15 surviving plants and stored in a non-air-conditioned glasshouse before being processed using a modified 'grist mill'. In March 2011, approximately 600 seeds from each of the two field-collected populations were planted into four replicate polystyrene foam trays (400 mm wide × 500 mm long × 150 mm deep) containing potting mixture (25% peat moss, 25% sand and 50% mulched pine bark). The known susceptible wild radish population WARR7 was collected in 1999 from a reserve at Yuna, Western Australia (latitude −28.34 S, longitude 115.01 E), where there had been no known herbicide application (referred to hereinafter as S1) (Table 1).[19] The transgenic glyphosate-resistant canola line (*Brassica napus* cv. RR Cobbler) was also included as a glyphosate-resistant control. At the two-true-leaf stage, seedlings were treated with glyphosate (Roundup Ready Herbicide™ 690 g glyphosate kg$^{-1}$; Nufarm, Laverton North, Victoria, Australia) at 540 g ha$^{-1}$. Plant survival was assessed at 42 days after treatment (DAT) by inspecting whether the rosette of each treated plant was actively growing.

### 2.2 General procedures

Plants were grown outdoors to simulate field conditions during their normal growing season at the University of Western Australia (Perth, WA). Plants were watered as required and fertilised weekly with 2 g of Scotts PolyFeed™ soluble fertiliser [N 19% (urea 15%, ammonium 1.9%, nitrate 2.1%), P 8%, K 16%, Mg 1.2%, S 3.8%, Fe 400 mg kg$^{-1}$, Mn 200 mg kg$^{-1}$, Zn 200 mg kg$^{-1}$, Cu 100 mg kg$^{-1}$, B 10 mg kg$^{-1}$, Mo 10 mg kg$^{-1}$]. Herbicide treatments were applied using a twin-nozzle laboratory sprayer fitted with 110° 01 flat-fan spray jets (Tee jet™) delivering herbicide in 100 L ha$^{-1}$ of water at 210 kPa, travelling at a speed of 3.6 km h$^{-1}$. The treated plants were returned outdoors, with survival assessed at 42 DAT. Survival was defined as plants that were actively growing.

### 2.3 Glyphosate dose response on field-collected populations

In June 2012, field-collected seeds of both putative glyphosate-resistant wild radish populations (R1 and R2) and the susceptible population (S1) (Table 1) were placed in 170 × 180 mm diameter plastic pots (20 seeds pot$^{-1}$) filled with standard potting mixture and maintained as previously described. At the two-true-leaf stage, all populations were sprayed with glyphosate (Roundup Ready Herbicide™ 690 g glyphosate kg$^{-1}$; Nufarm) at 0, 270, 540, 810, 1080, 1620 and 2160 g ha$^{-1}$. Survival was assessed at 42 DAT.

### 2.4 Glyphosate dose response on glyphosate-selected subpopulation

Thirty plants from each population, which survived glyphosate at rates ≥1080 g ha$^{-1}$, were subsampled and repotted into 350 × 305 mm diameter pots and isolated to prevent the ingress of foreign pollen. Once all individuals were flowering, plants were manually crossed using the Beestick method,[22] ensuring a random pattern of cross-pollination (panmixia). At maturity, all pods were collected and threshed using a modified 'grist mill', with the extracted seed representing the glyphosate-selected progeny subset of each population. In May 2013, the progeny of both populations (R1 and R2) and three known herbicide-susceptible wild radish populations S1, WARR33 (referred to hereinafter as S2)[20] and WARR36 (referred to hereinafter as S3) (Table 1) were seeded in 170 × 180 mm plastic pots (20 seeds pot$^{-1}$) filled with standard potting mixture and maintained as previously described. At the two-true-leaf stage, plants were sprayed with glyphosate (Roundup Ready Herbicide™ 690 g glyphosate kg$^{-1}$, Nufarm) at 0, 150, 300, 450, 600, 750, 900 and 1800 g ha$^{-1}$ for the susceptible control populations (S1, S2 and S3) and 0, 450, 750, 1050, 1500, 3000, 4500, 6000 and 7000 g ha$^{-1}$ for the glyphosate-selected resistant lines (R1 and R2). All populations were assessed for survival and biomass at 42 DAT.

### 2.5 Multiple resistance profile

Seedlings were sprayed with herbicides known to control wild radish (Table 2). In June 2013, 20 seeds from the original field-collected populations (R1 and R2) and the susceptible population (S1) were seeded into four replicate 170 × 180 mm plastic pots containing standard potting mixture. Plants were maintained as previously described and herbicide treated at the two-leaf-stage as per Table 2. Plant survival was assessed at 42 DAT.

**Table 1.** Location and date of collection of glyphosate-resistant and glyphosate-susceptible wild radish populations

| Designation | Population[a] | Glyphosate resistance status | Location collected | Year collected | Coordinates |
|---|---|---|---|---|---|
| S1 | WARR7 | Susceptible | Binnu, Western Australia | 1999 | 28.34° S, 115.01° E |
| S2 | WARR33 | Susceptible | Belmunging, Western Australia | 2004 | 31.88° S, 116.76° E |
| S3 | WARR36 | Susceptible | Carnac Island, Western Australia | 2013 | 32.12° S, 115.66° E |
| R1 | WARR37 | Resistant | Mingenew, Western Australia | 2010 | 29.11° S, 116.26° E |
| R2 | WARR38 | Resistant | Binnu, Western Australia | 2010 | 28.34° S, 115.01° E |

[a] WARR: Western Australian resistant radish.



  

**Table 2.** Multiple resistance traits in the field-collected (G0) wild radish populations R1 and R2 (zero indicates fully susceptible populations, 1–19% developing resistance and >20% survival results in classification as a resistant population). Standard errors are in parentheses

| Herbicide mode of action | Herbicide active ingredient | Rate (g ha$^{-1}$) | S1 mean percentage survival | R1 mean percentage survival[a] | R2 mean percentage survival[a] |
|---|---|---|---|---|---|
| ALS inhibitor | Chlorsulfuron[b] | 15 | 0 | 62 (4)* | 62 (5)* |
| ALS inhibitor | Metosulam[b] | 5 | 0 | 60 (2)* | 52(4)* |
| ALS inhibitor | Sulfometuron-methyl[b] | 7.5 | 0 | 55 (4)* | 58 (3)* |
| ALS inhibitor | Imazamox[b] | 32 | 0 | 22 (1)* | 47 (4)* |
| Synthetic auxin | 2,4-D amine | 500 | 1.19 (1.19) | 34 (4)* | 48 (5)* |
| Synthetic auxin | MCPA amine | 1000 | 0 | 10 (2) | 12 (2) |
| PDS inhibitor | Diflufenican | 100 | 4.10 (2.55) | 69 (9)* | 72 (2)* |
| PSII | Bromoxynil | 400 | 0 | 0 | 0 |
| PSII | Diuron | 1000 | 0 | 3 (3) | 2 (2) |
| PSII | Metribuzin | 280 | 0 | 5 (4) | 14 (5) |
| PSII | Atrazine[c] | 1000 | 0 | 0 | 0 |
| PSI | Diquat | 100 | 0 | 0 | 0 |

[a] An asterisk (*) denotes resistant (>20% survival).
[b] Plus BS 1000 nonionic surfactant.
[c] Plus Hasten crop oil concentrate.

### 2.6 Data analysis

The observed dose–response plant survival and biomass data were fitted to a three-parameter log-logistic model where the upper limit was fixed to 1.0 in R 3.0.0 (R Development Core Team 2011; http://www.R-project.org):[23,24]

$$Y = c + \left\{1 - c/(1 + \exp\left[b\left(\log x - \log e\right)\right]\right\} \quad (1)$$

where $Y$ denotes plant survival expressed as a percentage of the untreated control in response to herbicide dose $x$, $c$ is the lower asymptopic value of $Y$, $e$ is the estimated $LD_{50}$ or $GR_{50}$ and $b$ is the slope of the curve around the $LD_{50}$ or $GR_{50}$ parameter. The resistant (R) and susceptible (S) populations were compared using a resistant to susceptible (R/S) ratio of the estimated $LD_{50}$ values. The above-ground biomass was expressed as a percentage of the mean untreated control. Data were checked for homogeneity of variance, normality and independence of residuals, as described by Onofri *et al.*,[25] using a two-way analysis of variance (ANOVA) (Genstat v.6.1.0.200). Non-linear regression analysis was performed using the above three-parameter log-logistic model [equation (1)], and a *t*-test was used for statistical comparison of the means of each population curve using R 3.0.0 (R Development Core Team 2011; http://www.R-project.org). Data were plotted using SigmaPlot v.12 (Systat Software Inc., San Jose, CA, 2011).

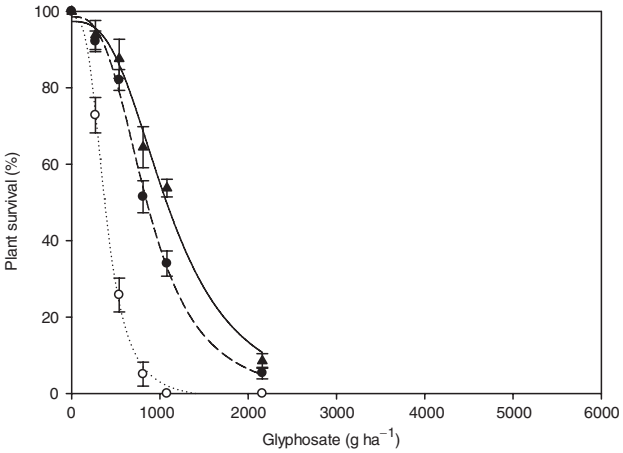


**Figure 1.** Survival dose–response curves for the susceptible wild radish population S1 (○) and field-collected glyphosate-resistant populations R1 (●) and R2 (▲). Each symbol represents the mean of five treatments for the susceptible population and six treatments for the resistant populations. The plotted lines are predicted survival curves using a three-parameter log-logistic model [equation (1)]. Vertical bars represent the mean ± SE ($n = 4$).

## 3 RESULTS AND DISCUSSION

### 3.1 Confirmation of glyphosate resistance

The results of these studies confirm the first glyphosate-resistant wild radish populations. Screening of the field-collected R1 and R2 populations at the recommended rate of glyphosate (540 g ha$^{-1}$) resulted in 63 and 86% survival respectively, while the known resistant control (*B. napus* cv. RR Cobbler) was unaffected and the known susceptible S1 population was killed. In subsequent dose–response studies with seed from survivors of field-collected populations, glyphosate resistance was quantified. The calculated R/S based on each population's $LD_{50}$ value indicated moderate resistance (Fig. 1; Table 3) and is comparable with the level of glyphosate resistance reported in *Amaranth* spp. (*A. tuberculatus* L. and *A. palmeri* L.),[26,27] giant ragweed (*Ambrosia trifida* L.),[28] fleabane (*C. bonariensis* L.),[29] ragweed plantain (*Parthenium hysteroporus* L.),[30] *Lolium* spp. (*L. rigidum* and *L. multiflorum* L.),[6,31] Johnson grass (*Sorghum halepense* L.)[32] and jungle rice (*E. colona* L.).[9]

Glyphosate resistance in R1 and R2 is inherited; however, the number of genes involved remains to be investigated. Progeny from plants surviving high glyphosate rates (≥1080 g ha$^{-1}$) increased survival (R1 73% and R2 92%) following screening with 750 g ha$^{-1}$, with all control populations (S1, S2 and S3) killed. This resulted in R/S at an $LD_{50}$ level of 3.5 (R1) and 4.5 (R2) when compared with the pooled values of the three susceptible populations (S1, S2 and S3) (Fig. 2; Table 3). Even though plants survived glyphosate application, their biomass was affected at higher rates, with the above-recommended glyphosate rate of 750 g ha$^{-1}$ reducing biomass in both populations (R1 16% and

**Table 3.** Parameter estimates for survival from the three-parameter log-logistic model [equation (1)] used to calculate $LD_{50}$ and R/S values for wild radish control populations (S1, S2 and S3) and the field-collected (G0) and the progeny subset glyphosate-resistant populations (G1) (R1 and R2) treated with a range of glyphosate doses (standard errors in parentheses)[a]

| Biotype | Population | *b* | *c* | *e* ($LD_{50}$) (g ha$^{-1}$) | R/S |
|---|---|---|---|---|---|
| | S1 | 3.15 (0.26) | −1.63 (1.78) | 384 (18) | – |
| G0 | R1 | 2.69 (0.25) | −1.45 (1.83) | 871 (35) | 2.3[b] |
| G0 | R2 | 2.82 (0.31) | −2.65 (2.43) | 1118 (66) | 3.2[b] |
| | S1 | 2.89 (0.31) | −6.24 (2.72) | 442 (28) | – |
| | S2 | 1.88 (0.23) | −9.02 (3.84) | 379 (45) | – |
| | S3 | 2.05 (0.22) | −8.92 (2.49) | 422 (40) | – |
| | Pooled control | 2.04 (0.3) | −9.38 (10.11) | 413 (12) | – |
| G1 | R1 | 1.82 (0.15) | −4.60 (2.49) | 1435 (131) | 3.5[c] |
| G1 | R2 | 1.99 (0.12) | 1.55 (2.05) | 1864 (136) | 4.5[c] |

[a] ANOVA analysis conducted on each non-linear regression is highly significant ($P < 0.001$).
[b] Resistant/susceptible ratios calculated between the base population S1 and field selected population.
[c] Resistant/susceptible ratios calculated between the parameters based upon the pooled susceptible and the selected F1 population.

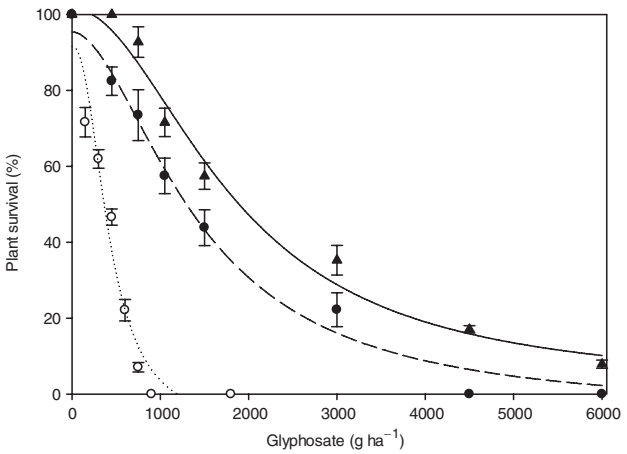


**Figure 2.** Survival dose–response curves for the pooled susceptible wild radish populations S1, S2 and S3 (.....○.....) and progeny subpopulation of glyphosate-resistant R1 (_ _●_ _) and R2 (__▲__). Each symbol represents the mean of seven treatments for the susceptible populations and eight treatments for the resistant populations. The plotted lines are predicted survival curves using a three-parameter log-logistic model [equation (1)]. Vertical bars represent the mean ± SE ($n = 5$).

R2 21% of the untreated), resulting in $GR_{50}$ rates of 207 g ha$^{-1}$ for pooled control populations (S1, S2 and S3), 260 g ha$^{-1}$ (R1) and 312 g ha$^{-1}$ (R2).

### 3.2 Evidence of multiple resistance

The glyphosate resistance evident in the R1 and R2 populations co-occurs with resistance to ALS-inhibiting, PDS-inhibiting and synthetic auxin herbicides (Table 2). Both populations are likely to contain multiple mechanisms conferring resistance to different herbicide modes of action.[33]

Overreliance on glyphosate without sufficient diversity of other weed management practices has been the prime cause of glyphosate resistance evolution worldwide.[34,35] Traditionally in Australia, glyphosate use has been limited to non-selective control of weeds prior to crop seeding or for fallow maintenance. This use pattern in combination with other modes of action has been considered to be sufficiently diverse to limit the widespread evolution of GR weeds.[34,35] Unfortunately, in the fields from which the R1 and R2 populations were collected, no detailed records of the herbicide use history could be obtained; therefore, no conclusion upon the sustainability of glyphosate use can be made.

Both glyphosate-resistant populations exhibited multiple resistance to three other herbicide modes of action. The evolution of multiple resistance incorporating glyphosate has been demonstrated in this cropping system in another genetically diverse species, annual ryegrass.[7] In the Americas there have also been reports of multiple resistance in glyphosate-resistant populations of *Amaranthus* spp. (*A. palmeri* and *A. tuberculatus* L.), *Ambrosia* spp. (*A. trifida* and *A. artenisiifolia* L.) and *Conyza* spp. (*C. canadensis* and *C. bonariensis* L.).[5] However, to the authors' knowledge, wild radish is only the second reported dicot species alongside waterhemp (*A. tuberculatus* L.) to exhibit resistance to four separate modes of action including glyphosate.[36] This multiple resistance is considered likely to have contributed to a reduction in the diversity of herbicidal control, probably leading to the selection of glyphosate-resistant biotypes.[35,37]

## 4 CONCLUSION

The multiple resistance, including glyphosate resistance, in two wild radish populations highlights the difficulty of herbicidal control of a resistance-prone species such as wild radish. The identification of these glyphosate-resistant wild radish populations is another example of the significant threat that glyphosate resistance and weed evolution pose to the sustainability of modern conservation farming systems. The mounting number of glyphosate-resistant weed populations and species demands the adoption of diverse weed control strategies. Glyphosate is most sustainable when weed control diversity is maximised.[35] This diversity may include alternating herbicide modes of action, mechanical control (tillage,[38] harvest weed seed control[39]) and biological options including maximising crop competition.[40,41]

## ACKNOWLEDGEMENTS

This research was funded by the Grains Research Development Corporation of Australia (GRDC) and the Australian Government through an Australian Postgraduate Award. The authors thank Craig Topham from Agrarian Management for supplying one location and AHRI staff who provided technical assistance.

## SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

## REFERENCES

1 Steinrücken HC and Amrhein N, The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimic acid-3-phosphate synthase. *Biochem Biophys Res Commun* **94**(4):1207–1212 (1980).
2 Amrhein N, Schab J and Steinrücken HC, The mode of action of the herbicide glyphosate. *Naturwissenschaften* **67**(7):356–357 (1980).
3 Amrhein N, Dues B, Gehrke P and Steinrucken H, The site of inhibition of the sikimate pathway of glyphosate. II. Interference of glyphosate with chorismate formation *in vivo* and *in vitro*. *Plant Physiol* **66**:830–834 (1980).

  

4 Green JM, Outlook on weed management in herbicide-resistant crops: need for diversification. *Outlooks Pest Manag* **22**(3):100–104 (2011).
5 Heap I, *The International Survey of Herbicide Resistant Weeds.* [Online]. Available: www.weedscience.com [20 February 2014].
6 Powles S, Lorraine-Colwill D, Dellow J and Preston C, Evolved resistance to glyphosate in rigid ryegrass (*Lolium rigidum*) in Australia. *Weed Sci* **46**:604–607 (1998).
7 Neve P, Sadler J and Powles SB, Multiple herbicide resistance in a glyphosate-resistant rigid ryegrass (*Lolium rigidum*) population. *Weed Sci* **52**(6):920–928 (2004).
8 Walker S, Bell K, Robinson G and Widderick M, Flaxleaf fleabane (*Conyza bonariensis*) populations have developed glyphosate resistance in north-east Australian cropping fields. *Crop Prot* **30**(3):311–317 (2011).
9 Gaines TA, Cripps A and Powles SB, Evolved resistance to glyphosate in junglerice (*Echinochloa colona*) from the tropical Ord River region in Australia. *Weed Technol* **26**(3):480–484 (2012).
10 Alemseged Y, Jones RE and Medd RW, A farmers' survey of weed management and herbicide problems on winter crops in Australia. *Plant Prot Q* **16**:21–25 (2001).
11 Borger CPD, Michael PJ, Mandel R, Hashem A, Bowran D and Renton M, Linking field and farmer surveys to determine the most important changes to weed incidence. *Weed Res* **52**(6):564–574 (2012).
12 Snow A and Campbell L, Can ferral radishes become weeds, in *Crop Ferality and Volunteerism*, ed. by Gressel J. CRC Press, Boca Raton, FL, pp. 193–207 (2005).
13 Reeves TG, Code GR and Piggin CM, Seed production and longevity, seasonal emergence and phenology of Wild Radish (*Rahanus raphanistrum*). *Aust J Exp Agric* **21**(112):524–530 (1981).
14 Eslami SV, Gill GS, Bellotti B and McDonald G, Wild radish (*Raphanus raphanistrum*) interference in wheat. *Weed Sci* **54**(4):749–756 (2006).
15 Blackshaw RE, Lemerle D and Young KR, Influence of wild radish on yield and quality of canola. *Weed Sci* **50**(3):344–349 (2002).
16 Cheam AH, Seed production and seed dormancy in wild radish (*Raphanus raphanistrum*) and some possibilities for improving control. *Weed Res* **26**(6):405–413 (1986).
17 Madhou P, Wells A, Pang E and Stevenson T, Genetic variation in populations of Western Australian Wild Radish. *Aust J Agric Res* **56**:1079–1087 (2005).
18 Hashem A, Bowran D, Piper T and Dhammu H, Resistance of wild radish (*Raphanus raphanistrum*) to acetolactate synthase-inhibiting herbicides in the Western Australia wheat-belt. *Weed Technol* **15**(1):68–74 (2001).
19 Walsh MJ, Powles SB, Beard BR and Porter SA, Multiple-herbicide resistance across four modes of action in wild radish (*Raphanus raphanistrum*). *Weed Sci* **52**(1):8–13 (2004).
20 Walsh MJ, Owen MJ and Powles SB, Frequency and distribution of herbicide resistance in *Raphanus raphanistrum* populations randomly collected across the Western Australian wheatbelt. *Weed Res* **47**(6):542–550 (2007).
21 Hashem A, Dhammu HS, Powles SB, Bowran DG, Piper TJ and Cheam AH, Triazine resistance in a biotype of wild radish (*Raphanus raphanistrum*) in Australia. *Weed Technol* **15**(4):636–641 (2001).
22 Williams PH, Beesticks, an aid in pollinating Cruciferae. *Hortscience* **15**(6):802–803 (1980).
23 Streibig JC, Rudemo M and Jensen JE, Dose–response curves and statistical models, in *Herbicide Bioassays*, ed. by Kudsk P and Streibig JC. CRC Press, Boca Raton, FL, pp. 29–55 (1993).
24 Price WJ, Shafii B and Seefeldt SS, Estimation of dose–response models for discrete and continuous data in weed science. *Weed Technol* **26**(3):587–601 (2012).
25 Onofri A, Carbonell EA, Piepho HP, Mortimer AM and Cousens RD, Current statistical issues in Weed Research. *Weed Res* **50**(1):5–24 (2010).
26 Zelaya IA and Owen MDK, Differential response of *Amaranthus tuberculatus* to glyphosate. *Pest Manag Sci* **61**(10):936–950 (2005).
27 Culpepper AS, Grey TL, Vencill WK, Kichler JM, Webster TM, Brown SM *et al.*, Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) confirmed in Georgia. *Weed Sci* **54**(4):620–626 (2006).
28 Norsworthy JK, Jha P, Steckel LE and Scott RC, Confirmation and control of glyphosate-resistant giant ragweed (*Ambrosia trifida*) in Tennessee. *Weed Technol* **24**(1):64–70 (2010).
29 VanGessel MJ, Glyphosate-resistant horseweed from Delaware. *Weed Sci* **49**(6):703–705 (2001).
30 Socorro J and Fuentes C, Resistencia de *Parthenium hysterophorus* L. al herbicida glifosato: un nuevo caso de resistencia a herbicidas en Colombia, in *2do Congreso Bianual SODIAF (Sociedad Dominicana de Investigadores Agropecuarios y Forestales)*, Santo Domingo, República Dominicana, p. 9 (2005).
31 Perez A and Kogan M, Glyphosate-resistant *Lolium multiflorum* in Chilean orchards. *Weed Res* **43**(1):12–19 (2003).
32 Vila-Aiub MM, Balbi MC, Gundel PE, Ghersa CM and Powles SB, Evolution of glyphosate-resistant Johnsongrass (*Sorghum halepense*) in glyphosate-resistant soybean. *Weed Sci* **55**:566–571 (2007).
33 Yu Q, Zhang XQ, Hashem A, Walsh MJ and Powles SB, ALS gene proline (197) mutations confer ALS herbicide resistance in eight separated wild radish (*Raphanus raphanistrum*) populations. *Weed Sci* **51**(6):831–838 (2003).
34 Preston C, Powles S, Wakelin A, Dolman F, Bostamam Y and Boutsalis P, A decade of glyphosate-resistant *Lolium* around the world: mechanisms, genes, fitness, and agronomic management. *Weed Sci* **57**(4):435–441 (2009).
35 Powles SB, Evolved glyphosate-resistant weeds around the world: lessons to be learnt. *Pest Manag Sci* **64**(4):360–365 (2008).
36 Bell MS, Hager AG and Tranel PJ, Multiple resistance to herbicides from four site-of-action groups in waterhemp (*Amaranthus tuberculatus*). *Weed Sci* **61**(3):460–468 (2013).
37 Neve P, Diggle AJ, Smith FP and Powles SB, Simulating evolution of glyphosate resistance in *Lolium rigidum* I: population biology of a rare resistance trait. *Weed Res* **43**(6):404–417 (2003).
38 Beckie HJ, Beneficial management practices to combat herbicide-resistant grass weeds in the Northern Great Plains. *Weed Technol* **21**(2):290–299 (2007).
39 Walsh M, Newman P and Powles S, Targeting weed seeds in-crop: a new weed control paradigm for global agriculture. *Weed Technol* **27**(3):431–436 (2013).
40 Beckie HJ, Johnson EN, Blackshaw RE and Gan YT, Weed suppression by canola and mustard cultivars. *Weed Technol* **22**(1):182–185 (2008).
41 Cousens RD, Warringa JW, Cameron JE and Hoy V, Early growth and development of wild radish (*Raphanus raphanistrum* L.) in relation to wheat. *Aust J Agric Res* **52**(7):755–769 (2001).




  