Research Article

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Response to low-dose herbicide selection in self-pollinated *Avena fatua*

Roberto Busi,* Marcelo Girotto and Stephen B Powles

Abstract

BACKGROUND: When applied at the correct plant stage and dose, herbicides are highly toxic to plants. At reduced, low herbicide doses (below the recommended dose) plants can survive and display continuous and quantitative variation in dose–survival responses. Recurrent (directional) selection studies can reveal whether such a phenotypic variation in plant survival response to low herbicide dose is heritable and leads to herbicide resistance. In a common experimental garden study, we have subjected a susceptible population of self-pollinated hexaploid *Avena fatua* to low-dose recurrent selection with the ACCase-inhibiting herbicide diclofop-methyl for three consecutive generations.

RESULTS: Significant differences in response to low-dose diclofop-methyl selection were observed between the selected progenies and parent plants, with a twofold diclofop-methyl resistance and cross-resistance to ALS-inhibiting herbicides. Thus, the capacity of self-pollinated *A. fatua* to respond to low-dose herbicide selection is marginal, and it is much lower than in cross-pollinated *L. rigidum*. *Lolium rigidum* in the same experiment evolved 40-fold diclofop-methyl resistance by progressive enrichment of quantitative resistance-endowing traits.

CONCLUSION: Cross-pollination rate, genetic variation and ploidy levels are identified as possible drivers affecting the contrasting capacity of *Avena* versus *Lolium* plants to respond to herbicide selection and the subsequent likelihood of resistance evolution at low herbicide dose usage.

Keywords: herbicide resistance; experimental evolution; low dose; reproductive system; self-pollination; cross-pollination

1 INTRODUCTION

When used at correct doses, modern crop-selective herbicides achieve high weed mortality without crop damage.[1] Globally each year, billions of weedy plant species succumb to herbicides, but under some conditions a low herbicide dose enables some survivors that reproduce, with evolutionary consequences. Targeted weedy plants can be exposed to a low, below-label herbicide dose, for example, if there is deliberate underdosing, tardy treatment time (oversized plants) or treatment under suboptimal environmental conditions. Most weedy plant species have significant genetic variability, including variability in response to herbicide dose.[2–10] Here, we are concerned with the evolutionary impacts of herbicide use at doses (low) that enable survivors that can reproduce.

ACCase-inhibiting herbicides (e.g. diclofop-methyl) are lethal to most grass weed species, whereas dicotyledonous species are mostly insensitive.[11] Introduced to world agriculture in the late 1970s, ACCase-inhibiting herbicides have been widely adopted for grass weed control in major crops. Cereal crops such as wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), rice (*Oryza sativa* L.) and barley (*Hordeum vulgare* L.) can effectively detoxify ACCase herbicides by metabolism, providing the basis for their selective in-crop use.[12,13]

Studies with the highly genetically variable, cross-pollinated and resistance-prone grass weed species *Lolium rigidum* (Gaud.) have established that recurrent selection at low herbicide dose can rapidly lead to herbicide resistance evolution.[4,14–18] Obligate cross-pollination in *L. rigidum* ensures that with low-dose herbicide selection the genetic traits conferring survival will be shared through cross-pollination, and herbicide resistance will evolve.[19] We expect this to be somewhat different in self-pollinated plant species, which, unlike cross-pollinated species, do not easily share genes. Many weedy plant species are self-pollinated, for example the resistance-prone global weed *Avena fatua* L.[20] In Australia and some other parts of the world, *Lolium* and *Avena* coinfest crop fields. Field selection often occurs with the same herbicides, and yet cross-pollinated *Lolium* has evolved resistance more rapidly, to a larger extent and with a greater number of modes of action than self-pollinated *Avena*, as reviewed elsewhere.[21–23]

In cross-pollinated *L. rigidum*, high-level resistance to the ACCase-inhibiting herbicide diclofop-methyl evolved after three generations of herbicide recurrent selection at below-label doses.[4,14] Here, we examine the potential for this to occur in a herbicide-susceptible population of self-pollinated hexaploid *A. fatua*. This provides information on the contribution of the reproductive system and the role of cross-pollination versus self-pollination in mediating the evolution of herbicide resistance.

* *Correspondence to: Roberto Busi, Australian Herbicide Resistance Initiative, School of Plant Biology, The University of Western Australia, 35 Stirling Highway, 6009 Crawley, WA, Australia. E-mail: roberto.busi@uwa.edu.au*

Australian Herbicide Resistance Initiative, School of Plant Biology, The University of Western Australia, Crawley, WA, Australia

Table 1. Herbicides and doses applied to unselected parents and diclofop-selected *Avena fatua* lines for cross-resistance characterisation

Mode of action	Herbicide active ingredient	Labelled use rate (g ha^{-1})	Dose applied (g ha^{-1})
ACCase inhibitor	Clodinafop	18	18
ACCase inhibitor	Diclofop-methyl	600	375
ACCase inhibitor	Pinoxaden	20	20
ACCase inhibitor	Sethoxydim	186	186
ALS inhibitor	Mesosulfuron	10	10
ALS inhibitor	Imazapyr + imazapic	10.5 + 3.5	10.5 + 3.5
EPSPS inhibitor	Glyphosate	450	150, 250
PSI electron diversion	Paraquat	200	100

2 MATERIALS AND METHODS

2.1 Plant material

Seeds of a known herbicide-susceptible *A. fatua* population were collected in December 2004 from approximately 100 mature plants growing in a natural Perth bushland area (31° 56′ 41.24″ S, 115° 46′ 28.23″ E). This area is located more than 100 km from cropping regions and has no history of herbicide use.

2.2 Recurrent selection with the herbicide diclofop-methyl

Cycles of recurrent selection by low diclofop-methyl dose were performed with the herbicide-susceptible *A. fatua* population, as detailed in Table 1. This selection study was conducted over three consecutive years with plants grown in the normal southern Australian winter growing season (May–August) in a natural outdoor experimental garden simulating field conditions. In each growing season, one selection cycle was performed by treating *A. fatua* at low herbicide dose and maintaining survivors through seed production in late spring. Seed was collected from survivors, stored in dry conditions over summer and germinated in the following growing season and subjected to the next round of low-dose diclofop-methyl selection.

2.3 Herbicide dose–response assays

Seeds were germinated on 0.6% water-solidified agar and 2 cm emerging seedlings transplanted into 2 L pots containing a commercial potting soil mix (50% peat moss, 25% sand and 25% pine bark). The susceptible *A. fatua* population was selected at low dose (below the recommended label dose) of diclofop-methyl (ACCase-inhibiting herbicide). Diclofop-methyl was applied at 0, 375 and 600 g ha^{-1} to two-leaf-stage seedlings. There were at least four replications per treatment dose, and at least 17 seedlings per replication (pot). Each pot represented the experimental unit. Plants were kept well watered (>80% field capacity) and fertilised. At 28 days after herbicide treatment, plant survival was recorded. Individuals that survived 375 g diclofop-methyl ha^{-1} were transplanted into 10 L pots (5 plants pot^{-1}) and at flowering were confined in pollen-proof enclosures to avoid any pollen contamination, and then mature seeds were collected. The seed obtained from these selected plants represented the selected bulk-crossed progeny. The following season the selected progeny was subjected to the next cycle of low-dose diclofop-methyl selection. This process was repeated over three consecutive generations (for details, see Table 1).

2.4 Final diclofop-methyl dose–response study

After three cycles of low-dose diclofop-methyl selection experiment, the initial herbicide-susceptible *A. fatua* parent and all the respective 1×, 2× and 3× selected progenies were evaluated in herbicide dose–response experiments under identical growing conditions. At the two-leaf stage, seedlings were diclofop-methyl treated (0, 188, 375, 600 or 1200 g ha^{-1}). After 28 days, survival assessments were made, plants were cut at the soil surface above the meristematic zone and the harvested material was oven-dried to determine above-ground plant biomass. For each herbicide dose there were three replicates and 17 plants per replicate. This final dose–response study was repeated.

2.5 Cross-resistance endowed by diclofop-methyl recurrent selection

We examined for any evidence of cross-resistance to four ACCase-inhibiting herbicides (clodinafop, diclofop-methyl, pinoxaden and sethoxydim), two ALS-inhibiting herbicides (mesosulfuron and imazapyr + imazapic) and two non-selective herbicides (glyphosate and paraquat). The diclofop-methyl-selected and unselected lines were treated with herbicides at the two-leaf stage at rates as shown in Table 1. Plant survival was assessed 28 days after herbicide treatments.

2.6 Statistical analysis

Data from the two final experiments were pooled because there was no significant difference in the response of the controls (unselected parental populations) in the two repeated experiments. Plant survival values were obtained by dividing the number of survivors by the total number of treated plants per replicate. Survival values ranged between 0 and 1, and a binomial distribution of errors was adopted in the non-linear regression analysis. Plant growth was calculated as above-ground biomass values as a percentage of the untreated control and was analysed with the same model assuming a Gaussian continuous distribution of errors in non-linear regression analysis. The herbicide doses causing 50% plant mortality (LD_{50}) or growth reduction (GR_{50}) in the selected and unselected populations at each generation were estimated by using a three-parameter log-logistic model:

$$Y = \frac{d}{1 + exp\left[b\left(logx - loge\right)\right]} \quad (1)$$

where d is the upper limit, b is the slope of the curve, x is the herbicide dose and e is the dose producing a 50% reduction in response. The response to selection in the selected progenies was measured as the resistance index (RI). RI is the ratio between selected and unselected and here is defined as the ratio of estimated LD_{50} or GR_{50} values between each selected progeny and the unselected parental population. The statistical difference in estimated LD_{50} or GR_{50} values of the each selected progeny and the unselected parental population was assessed by using the selectivity index (SI) function in the *drc* package of the statistical software R v.2.11.1 (2010; R Foundation for Statistical Computing, Vienna, Austria).

Cross-resistance was quantified by assessing the differences in survival between unselected parents and selected progenies with diclofop-methyl in response to different herbicide modes of action, as detailed in Table 2. Survival data values were subjected to chi-square analysis. The null hypothesis H_0 was that the expected survival for any herbicide mode of action tested would

Table 2. Seed progeny collected from a number of selected survivors (*Ni*) for a specific low dose of diclofop-methyl (g ha^{-1}) resulting in mean selection intensity (survival %). *Avena fatua* populations exposed to herbicide selection with diclofop for up to three generations. Estimated LD_{50} and GR_{50} values and their R/S ratios (resistance index) are reported for selected lines and unselected parents (generation 0) from dose–response studies. Probability values (*P*) of difference between the LD_{50} or GR_{50} of the unselected parental WA population and the respective selected progeny calculated by selectivity index (SI) function in the *drc* package of the statistical software R v.2.11.1 (2010)

Herbicide	Population	Generation	Dose applied (g ha^{-1})	Selection intensity (survival %)	Plants selected (*Ni*)	LD_{50} (g ha^{-1})	*P*	GR_{50} selected/ parent	*P*
–	Parent	0	–	–	–	238 (25)	–	145 (25)	–
Diclofop	Progeny 1	1	375	0.53	24	463 (53)	<0.001	157 (31)	0.742
Diclofop	Progeny 2	2	375	0.12	11	453 (34)	<0.001	194 (28)	0.125
Diclofop	Progeny 3	3	375	0.44	20	546 (49)	<0.001	191 (24)	0.131

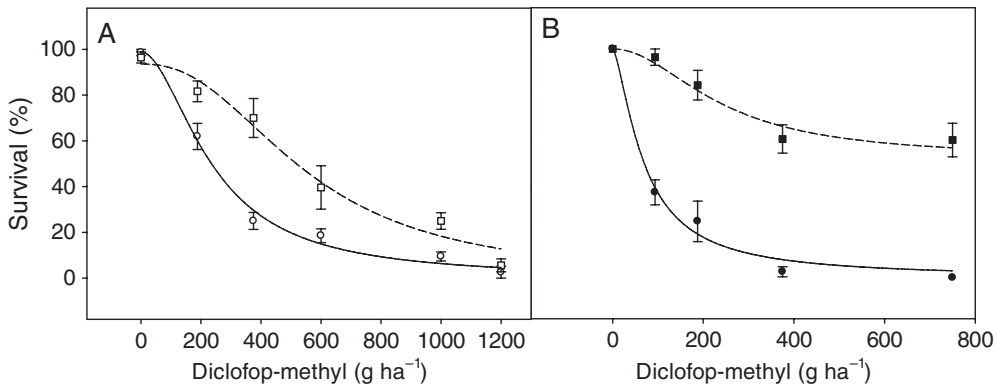

Figure 1. Response to low-dose diclofop-methyl recurrent selection in *A. fatua* (A) versus *L. rigidum* (B). The 3× selected seed progenies and the unselected original parental populations were compared by a herbicide dose–response study. – -o- – *A. fatua* parental line (WA); –-□-- progeny selected 3 times with diclofop-methyl at 375 g diclofop-methyl ha^{-1} (WA-P3); – -●- – *L. rigidum* parental line (VLR1); – -■- – progeny selected 3 times with diclofop-methyl (VLR1 0.1, 0.5, 2.0). Symbols are observed means ± SE ($n = 6$). Lines represent the fitted logistic model [equation (1)]. Graph (B) is redrawn from data obtained by Neve and Powles.[14]

have been the same across all unselected and selected lines. Multiple comparisons among survival proportions of selected and unselected lines were assessed by χ^2 heterogeneity test performed using the statistical software *R* with the command *prop.test*.

3 RESULTS

In several repeated diclofop-methyl dose–response assays it was established that the unselected *A. fatua* parent population, collected from an area with no previous herbicide exposure history, was susceptible to diclofop-methyl (data not shown).

3.1 Response to low-dose diclofop-methyl recurrent selection

As expected, when the herbicide-susceptible *A. fatua* parent population was treated at a low, below-label dose of 375 g diclofop-methyl ha^{-1} there were around 50% survivors (Table 1). In order to establish whether these individuals survived this low diclofop-methyl dose owing to heritable genetic traits, these survivors were isolated, and they self-pollinated to produce seed termed the 1× diclofop-methyl-selected line. This same recurrent selection process was repeated in year 2 to produce the 2× diclofop-methyl-selected seed progeny, which was subjected to the same process in year 3 to produce the 3× diclofop-methyl-selected seed progeny (three consecutive generations). Between years, only small differences in diclofop-methyl efficacy were observed (Table 1), which can probably be explained by the slightly different environmental conditions prevailing during selection over the three consecutive years. Finally, in one large herbicide dose–response study, the unselected herbicide-susceptible parents were compared with the 1×, 2× and 3× diclofop-methyl-selected lines. A shift in herbicide susceptibility was evident. The 3× recurrently selected generation survived at greater percentages across a full range of diclofop-methyl doses (Fig. 1A). For example, at the recommended label dose of 600 g diclofop ha^{-1}, the susceptible parental line exhibited 18% survival, whereas progeny 3 showed 40% survival. The population parameters LD_{50} (50% population lethal dose) and GR_{50} (50% growth reduction) values were obtained from detailed dose–response studies to quantify the results of 3 years of low-dose diclofop-methyl selection in *A. fatua* populations (Table 2). This initially herbicide-susceptible population evolved only a modest level of diclofop-methyl resistance over three generations, with a calculated resistance LD_{50} index ratio of 2.3 (Table 3). There was a 1.3-fold increase in GR_{50} values between the 3× selected progeny and the unselected parent population ($P > 0.125$) (Table 2). It is important to highlight that this modest level of evolved diclofop-methyl resistance in self-pollinated *A. fatua* is in marked contrast to the high-level resistance that was observed in cross-pollinated *L. rigidum* in an identical experimental garden recurrent selection study (Fig. 1B and Table 3).

3.2 Evidence of herbicide cross-resistance

Eight different herbicides of four different modes of action were used to quantify any cross-resistance shift in diclofop-selected progenies (Table 2). There was greater survival to the ALS herbicides mesosulfuron and imazapyr + imazapic in plants selected at low dose of diclofop-methyl than in the unselected parent

Table 3. Response to selection with low dose of diclofop-methyl in cross-pollinated *Lolium rigidum* versus self-pollinated *Avena fatua*. The LD_{50} R/S (resistance index) is the calculated ratio of the estimated LD_{50} value of selected progeny and the LD_{50} of the unselected parental line

Reproductive mode	Genus	Herbicide selected agent	Selection dose (mean)	Progeny	LD_{50}	LD_{50} ratio selected/ unselected
Self-pollinated	*Avena*	Diclofop	Unselected	0	238	–
Self-pollinated	*Avena*	Diclofop	375	3[a]	546	2.3
Cross-pollinated	*Lolium*	Diclofop	Unselected	0	61[c]	–
Cross-pollinated	*Lolium*	Diclofop	325	3[b]	2462[c]	40

[a] Progeny selected with three cycles of recurrent selection from the parental WA herbicide-susceptible *A. fatua* population, as detailed in Table 1. Herbicide selection occurred at 375 g diclofop ha^{-1} as 0.66× the recommended label dose (labelled dose 600 g diclofop ha^{-1}).
[b] Progeny selected with three cycles of recurrent selection from the parental herbicide-susceptible *L. rigidum* population VLR1. Herbicide selection occurred at 37.5, 188 and 750 g diclofop ha^{-1} as 0.1×, 0.5× and 2.0× the recommended label dose (labelled dose 375 g diclofop ha^{-1}).[14]
[c] Data reported by Neve and Powles.[14]

($P < 0.0026$) (Table 4). Thus, there was some evidence of a moderate shift towards cross-resistance to the ALS-inhibiting herbicides in low-dose diclofop-methyl-selected *A. fatua*. No differences in plant survival between parent and progeny plants were recorded in response to any other herbicide tested (Table 4).

4 DISCUSSION

In this study we characterised the capability of a small herbicide-susceptible population of self-pollinated *A. fatua* to respond to recurrent low-dose selection with the herbicide diclofop-methyl, and we found that a moderate level of diclofop-methyl resistance evolved after three selection cycles. By contrast, in cross-pollinated *L. rigidum* subjected to the same experimental protocol of low-dose diclofop-methyl selection, we observed a high-level diclofop-methyl resistance in the low-dose selected progeny (Fig. 1). Thus, in cross-pollinated *L. rigidum*, diclofop-methyl resistance following low-dose recurrent selection was 20-fold greater than in self-pollinated *A. fatua* (Table 3). It is likely that there are minor genetic traits present in both unselected parent herbicide-susceptible *A. fatua* and *L. rigidum* that can contribute to survival at low dose of diclofop-methyl. For example, diclofop-methyl is known to be metabolised at a low rate by both herbicide-susceptible *Lolium* and *Avena* plants.[13,24,25] However, it appears that biological traits of these two grass species determined a considerably different evolutionary outcome.

Low herbicide doses (herbicide selection acting within standing genetic variation of herbicide-susceptible plants) allow any trait contributing to a marginally greater plant survival and growth to be inherited in the selected progeny.[26] Thus, individuals with marginally greater capacity for degradative metabolism of diclofop-methyl (and other metabolisable herbicides) survive the low dose, probably through constitutive increased expression of several minor P450 genes or other functionally relevant enzymes, as reviewed elsewhere.[19] In *L. rigidum*, a series of studies have demonstrated the polygenic nature of low-dose selected diclofop-methyl resistance and cross-resistance to ALS herbicides,[26] the enhanced capacity of diclofop-methyl-resistant *L. rigidum* progeny plants to metabolise diclofop-methyl after exposure to low-dose selection[24] and the likely involvement of enzyme superfamilies such as cytochrome P450 monooxygenases and GST.[27] Gaines *et al.*[27] clearly reveal that a great diversity of minor traits for herbicide metabolism exist in unselected herbicide-susceptible *L. rigidum* parent plants at a relatively high frequency, yet individually these minor resistance-endowing genes (i.e. GT, P450s), may not endow a sufficient level of resistance at the recommended label dose of the herbicide diclofop-methyl. However, starting with herbicide-susceptible parent plants, when these minor traits are selected, enriched and inherited in the progeny with a few generations of low-dose diclofop-methyl selection, high-level resistance rapidly occurs.[4,14,18]

Thus, cross-pollination appears to be effective in enabling resistance-endowing gene recombination and accumulation, whereas self-pollination can limit such a progressive response to selection. In this study we have not directly measured the outcrossing rate in *A. fatua*, as it is well known that this species is almost completely self-pollinated, with an estimated outcrossing rate of <1%,[20,28] and when recurrently selected for three generations at low diclofop-methyl dose it evolves a modest level of resistance to diclofop-methyl. Very long-term recurrent selection studies (100 years) have shown that cross-pollinated plant species can retain a significant proportion of the overall genetic variation for traits under selection.[29] Conversely, self-pollination by limiting genetic recombination in the selected progeny could also contribute to the erosion of the genetic variation of a selected trait.[30–32] In this selection study, the *A. fatua* population under selection was small, with a limited number of plants herbicide selected at each generation. It is possible that a greater effective population size in *A. fatua* could have counterbalanced a limited genetic exchange, favoured recombination of favourable alleles among the few individuals selected and limited the subsequent decline of selectable genetic variation.[31] Finally, the different level of ploidy in *Lolium* versus *Avena* could also have contributed to the final shift in herbicide resistance observed in the two species. Polyploid *Avena* is more adapted to different environments than its diploid ancestors.[33] Thus, we expected hexaploid *A. fatua* to show adaptive potential to low-dose herbicide recurrent selection, as previously observed in different *L. rigidum* populations.[4,14,18] However, in diploid *Lolium* a single functional mutant allele in the target gene, depending on the genetic dominance of the specific gene mutation, can confer substantial phenotypic resistance.[34,35] Conversely, in hexaploid *Avena* the same functional gene mutations endowing herbicide resistance (i.e. ACCase Ile-1781-Leu, Asp-2078-Gly and Cys-2088-Arg) result in a lower-than-expected level of phenotypic resistance, probably because susceptible ACCase (the target enzyme for ACCase herbicides) is present in homoeologs of hexaploid *Avena* plants and constitutively expressed.[36] Thus, it is possible that the modest diclofop-methyl

Table 4. Pooled plant survival of unselected parent WA *A. fatua* versus herbicide-selected lines (pooled). Inferences on evolved cross-resistance were made by comparing the survival ratio (surviving/treated plants) in selected versus unselected lines by chi-square analysis. The *P*-values indicate the probability of type 1 error in rejecting the null hypothesis (null hypothesis = no difference in survival between parental and herbicide-selected lines)

Herbicide	Herbicide dose (g ha^{-1})	Line(s)	Plants treated	Survivors	Survival (%)	χ^2	*P*
Clodinafop	18	Selected	59	0	0	0.241	0.623
Clodinafop	18	Unselected parent	23	1	4.3	–	–
Pinoxaden	20	Selected	49	0	0	0.105	0.745
Pinoxaden	20	Unselected parent	26	1	3.8	–	–
Sethoxydim	186	Selected	67	3	4.4	0.295	0.587
Sethoxydim	186	Unselected parent	30	0	0	–	–
Mesosulfuron	10	Selected	66	41	62	9.1	0.0026
Mesosulfuron	10	Unselected parent	25	6	24	–	–
Imazapyr + imazapic	10.5 + 3.5	Selected	82	58	70.7	9.41	0.0025
Imazapyr + imazapic	10.5 + 3.5	Unselected parent	28	10	35.7	–	–
Glyphosate	150	Selected	177	69	39	0.0036	0.952
Glyphosate	150	Unselected parent	51	19	37	–	–
Glyphosate	250	Selected	180	17	9.4	0.088	0.766
Glyphosate	250	Unselected parent	69	5	7.2	–	–
Paraquat	100	Selected	66	0	0	0.197	0.6568
Paraquat	100	Unselected parent	28	1	3.6	–	–

resistance selected in *A. fauta* was due to its ploidy level decreasing the rate of minor resistance gene accumulation (i.e. a dilution effect of homoeologs). However, this hypothesis remains to be tested.

In conclusion, we have demonstrated that low-dose herbicide selection resulted in low-level resistance (and cross-resistance) in self-pollinated *Avena*. In small populations, self-pollination can greatly limit the response to low-dose herbicide selection, thus reducing the potential for adaptation of a weed species. Herbicide resistance and cross-resistance endowed by an enhanced capacity for herbicide detoxification have evolved in many different species, regardless of the reproductive mode, as reviewed elsewhere.[19,23,37,38] However, this study suggests that the reproductive system is an important factor in herbicide resistance evolution, especially when weed control is suboptimal owing to the use of a low herbicide dose or other environmental reasons. As observed in other systems,[39] we speculate that cross-pollination, with some marked differences among species, can increase the likelihood of herbicide resistance evolution in weed species by efficient minor resistance trait accumulation. This can be critical in the initial stages of weed resistance selection by preventing population extinction through evolutionary rescue.

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REFERENCES

1 Kraehmer H, Laber B, Rosinger C and Schulz A, Herbicides as weed control agents: state of the art: I. Weed control research and safener technology: the path to modern agriculture. *Plant Physiol* **166**:1119–1131 (2014).
2 Holliday RJ and Putwain PD, Evolution of herbicide resistance in *Senecio vulgaris*: variation in susceptibility to simazine between and within populations. *J Appl Ecol* **17**:779–791 (1980).
3 Gillespie GR and Vitolo DB, Response of quackgrass (*Elytrigia repens*) biotypes to primisulfuron. *Weed Technol* **7**:411–416 (1993).
4 Neve P and Powles S, High survival frequencies at low herbicide use rates in populations of *Lolium rigidum* result in rapid evolution of herbicide resistance. *Heredity* **95**:485–492 (2005).
5 Huangfu CH, Song XL, Qiang S and Zhang HJ, Response of wild *Brassica juncea* populations to glyphosate. *Pest Manag Sci* **63**:1133–1140 (2007).
6 Norsworthy JK, Scott RC, Smith KL and Oliver LR, Response of northeastern Arkansas Palmer Amaranth (*Amaranthus palmeri*) accessions to glyphosate. *Weed Technol* **22**:408–413 (2008).
7 Patzoldt WL, Tranel PJ and Hager AG, Variable herbicide responses among Illinois waterhemp *Amaranthus rudis* and *A. tuberculatus* populations. *Crop Prot* **21**:707–712 (2002).
8 Paterson EA, Shenton ZL and Straszewski AE, Establishment of the baseline sensitivity and monitoring response of *Papaver rhoeas* populations to florasulam. *Pest Manag Sci* **58**:964–966 (2002).
9 Escorial C, Loureiro I, Rodríguez-García E and Chueca C, Population variability in the response of ripgut brome (*Bromus diandrus*) to sulfosulfuron and glyphosate herbicides. *Weed Sci* **59**:107–112 (2011).
10 Vidotto F, Tesio F, Tabacchi M and Ferrero A, Herbicide sensitivity of *Echinochloa* spp. accessions in Italian rice fields. *Crop Prot* **26**:285–293 (2007).
11 Burton JD, Gronwald JW, Somers DA, Connelly JA, Gengenbach BG and Wyse DL, Inhibition of plant acetyl-coenzyme a carboxylase by the herbicides sethoxydim and haloxyfop. *Biochem Biophys Res Commun* **148**:1039–1044 (1987).
12 Konishi T and Sasaki Y, Compartmentalization of two forms of acetyl-CoA carboxylase in plants and the origin of their tolerance toward herbicides. *Proc Natl Acad Sci* **91**:3598–3601 (1994).
13 Shimabukuro RH, Walsh WC and Hoerauf RA, Metabolism and selectivity of diclofop-methyl in wild oat and wheat. *J Agric Food Chem* **27**:615–623 (1979).
14 Neve P and Powles S, Recurrent selection with reduced herbicide rates results in the rapid evolution of herbicide resistance in *Lolium rigidum*. *Theor Appl Genet* **110**:1154–1166 (2005).
15 Busi R, Gaines TA, Walsh MJ and Powles SB, Understanding the potential for resistance evolution to the new herbicide pyroxasulfone: field selection at high doses versus recurrent selection at low doses. *Weed Res* **52**:489–499 (2012).
16 Busi R and Powles SB, Evolution of glyphosate resistance in a *Lolium rigidum* population by glyphosate selection at sub-lethal doses. *Heredity* **103**:318–325 (2009).

17 Busi R and Powles SB, Reduced sensitivity to paraquat evolves under selection with low glyphosate doses in *Lolium rigidum*. *Agron Sustain Dev* **31**:525–531 (2011).
18 Manalil SV, Busi R, Renton M and Powles SB, Rapid evolution of herbicide resistance by low herbicide dosages. *Weed Sci* **59**:210–217 (2011).
19 Yu Q and Powles S, Metabolism-based herbicide resistance and cross-resistance in crop weeds: a threat to herbicide sustainability and global crop production. *Plant Physiol* **166**:1106–1118 (2014).
20 Beckie HJ, Francis A and Hall LM, The biology of Canadian weeds. 27. *Avena fatua* L. (updated). *Can J Plant Sci* **92**:1329–1357 (2012).
21 Owen MC, Walsh MJ, Llewellyn RS and Powles SB, Widespread evolution of multiple herbicide resistance in annual ryegrass (*Lolium rigidum*) populations within the Western Australian wheat belt. *Aust J Agric Res* **58**:711–718 (2007).
22 Owen MJ and Powles SB, Distribution and frequency of herbicide-resistant wild oat (*Avena* spp.) across the Western Australian grain belt. *Crop Past Sci* **60**:25–31 (2009).
23 Busi R, Resistance to herbicides inhibiting the biosynthesis of very-long-chain fatty acids. *Pest Manag Sci* accepted (2014).
24 Yu Q, Han H, Cawthray GR, Wang SF and Powles SB, Enhanced rates of herbicide metabolism in low herbicide-dose selected resistant *Lolium rigidum*. *Plant Cell Environ* **36**:818–827 (2013).
25 Ahmad-Hamdani M, Yu Q, Han H, Cawthray GR, Wang SF and Powles SB, Herbicide resistance endowed by enhanced rates of herbicide metabolism in wild oat (*Avena* spp.). *Weed Sci* **61**:55–62 (2013).
26 Busi R, Neve P and Powles S, Evolved polygenic herbicide resistance in *Lolium rigidum* by low-dose herbicide selection within standing genetic variation. *Evol Applic* **6**:231–242 (2013).
27 Gaines TA, Lorentz L, Figge A, Herrmann J, Maiwald F, Ott MC *et al.*, RNA-Seq transcriptome analysis to identify genes involved in metabolism-based diclofop resistance in *Lolium rigidum*. *Plant J* **78**:865–876 (2014).
28 Murray BG, Morrison IN and Friesen LF, Pollen-mediated gene flow in wild oat. *Weed Sci* **50**:321–325 (2002).
29 Dudley JW and Lambert RJ, 100 generations of selection for oil and protein in corn. *Plant Breed Rev* **24**:79–110 (2004).
30 Robertson A, Selection for heterozygotes in small populations. *Genetics* **47**:1291–1300 (1962).
31 David JL, Savy Y and Brabant P, Outcrossing and selfing evolution in populations under directional selection. *Heredity* **71**:642–651 (1993).
32 Allard RW, The mating system and microevolution. *Genetics* **79**:115–126 (1975).
33 Garcia P, Morris M, Saenz-de-Miera L, Allard R, de la Vega MP and Ladizinsky G, Genetic diversity and adaptedness in tetraploid *Avena barbata* and its diploid ancestors *Avena hirtula* and *Avena wiestii*. *Proc Natl Acad Sci* **88**:1207–1211 (1991).
34 Kaundun SS, Bailly GC, Dale RP, Hutchings S-J and McIndoe E, A novel W1999S mutation and non-target site resistance impact on Acetyl-CoA carboxylase inhibiting herbicides to varying degrees in a UK *Lolium multiflorum* population. *PLoS ONE* **8**:e58012 (2013).
35 Kaundun SS, An aspartate to glycine change in the carboxyl transferase domain of acetyl CoA carboxylase and non-target-site mechanism(s) confer resistance to ACCase inhibitor herbicides in a *Lolium multiflorum* population. *Pest Manag Sci* **66**:1249–1256 (2010).
36 Yu Q, Ahmad-Hamdani MS, Han H, Christoffers MJ and Powles SB, Herbicide resistance-endowing ACCase gene mutations in hexaploid wild oat (*Avena fatua*): insights into resistance evolution in a hexaploid species. *Heredity* **110**:220–231 (2013).
37 Beckie HJ and Tardif FJ, Herbicide cross resistance in weeds. *Crop Prot* **35**:15–28 (2012).
38 Sammons RD and Gaines TA, Glyphosate resistance: state of knowledge. *Pest Manag Sci* (2014).
39 Lagator M, Morgan A, Neve P and Colegrave N, Role of sex and migration in adaptation to sink environments. *Evolution* **68**:2296–2305 (2014).

