Research Article

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Glyphosate resistance in *Echinochloa colona*: phenotypic characterisation and quantification of selection intensity

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Abstract

BACKGROUND: A population of *Echinochloa colona* infesting agricultural fields in the northern region of Western Australia evolved glyphosate resistance after 10 years of glyphosate selection. This study identified two phenotypic (susceptible S versus resistant R) lines from within a segregating glyphosate-resistant population. Estimation of survival, growth and reproductive rates of the phenotypes in response to glyphosate selection helped to characterise the level of resistance, fitness and the selection intensity for glyphosate in this species.

RESULTS: Estimations of LD_{50} (lethal dose) and GR_{50} (growth rate) showed an eightfold glyphosate resistance in this population. The resistant index based on the estimation of seed number (SY_{n50}) showed a 13-fold resistance. As a result of linear combination of plant survival and fecundity rates, plant fitness values of 0.2 and 0.8 were estimated for the S and R phenotypes when exposed to the low dose of 270 g glyphosate ha^{-1}. At the recommended dose of 540 g glyphosate ha^{-1}, fitness significantly decreased (fivefold) in S plants but remained markedly similar (0.7) in plants of the R phenotype. Thus, the calculated selection intensity (SI) at 540 g glyphosate ha^{-1} was much greater (SI = 17) than at 270 g glyphosate ha^{-1} (SI = 4).

CONCLUSIONS: The assessment of plant survival and fecundity in response to glyphosate selection in the S and R phenotypes allowed a greater accuracy in the estimation of population fitness of both phenotypes and thus of glyphosate selection intensity in *E. colona*. The estimation of seed number or mass of phenotypes under herbicide selection is a true ecological measure of resistance with implications for herbicide resistance evolution.

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

Keywords: glyphosate resistance; selection intensity; *Echinochloa colona*; fitness; fecundity

1 INTRODUCTION

Herbicide resistance alleles are rare traits in plants, and very few studies have attempted to estimate the initial frequency of resistance.[1–3] However, continuous herbicide selection often leads to adaptive evolution towards herbicide resistance.[4] Herbicide resistance evolution occurs in agricultural landscapes as well as recreation areas, roadsides and railway lines where herbicide selection occurs.[5–7]

Herbicide resistance is an evolutionary process wh ere survival and reproduction (i.e. fitness) of individuals with resistance alleles in a population are enriched in the presence of the herbicide.[4] The population dynamics and enrichment rate of resistance alleles in populations are greatly influenced by genetic factors (gene mutation rate, dominance, additivity, epistasis, pleiotrophy, inheritance mode, ploidy) and biological factors (reproduction and mating system, population size, number of generations)[2,8] and by environmental conditions.[9,10]

Herbicide bioassays are the standard experimental protocol to diagnose resistance in weed species at the whole-plant level.[11,12] These studies are useful as they estimate survival and/or growth of susceptible (S) and resistant (R) populations under increasing herbicide selection doses. This approach enables the estimation of resistance indexes consisting of the ratio (R/S) of population parameters such as LD_{50} (lethal dose) and GR_{50} (growth rate). However, survival rates in herbicide-exposed populations do not provide information about the evolutionary dynamics of a resistance-endowing trait in a weed population under herbicide selection.

The estimation of both survival and fecundity rates (e.g. fitness) in resistant genotypes when exposed to herbicides are a true ecological measure of resistance. Assessment of plant fitness in

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response to herbicide enables the quantification of the selection intensity of resistance, a parameter that accounts for the relative resistance level of a genotype to a particular herbicide and dose and thus enables the estimation of the frequency changes of resistance genes under selection. In spite of its importance, very few studies have empirically estimated the intensity of herbicide selection (SI).[13,14] For example, Beckie and Morrison[14] estimated a 29-fold selective advantage of trifluralin-resistant plants compared with trifluralin-susceptible plants treated at the full recommended dose. This requires the estimation of both plant survival and fecundity of S and R phenotypes under herbicide selection,[8] ensuring a common genetic background.[15,16]

Diagnosis of glyphosate resistance has been confirmed in 31 weed species[17] representing a current threat to agriculture sustainability. *Echinochloa colona* (awnless barnyardgrass), a C_4 annual species, has evolved glyphosate resistance when infesting summer crops in Argentina, Australia and the United States.[18,19]

In this study we characterise the glyphosate resistance level, population fitness and selection intensity after assessing the survival, growth and reproductive rates of S and R *E. colona* phenotypes collected in Western Australia. We adopt an often overlooked methodological protocol and discuss the results towards an improved prediction of glyphosate resistance evolution in *E. colona* and other species.[20,21]

2 MATERIALS AND METHODS

2.1 Plant material

Seed samples of *E. colona* were collected in early Autumn 2010 from a 32 ha watermelon crop field in the Tropical Ord River region (15° 30′ S, 128° 21′ E) of Western Australia. The field had received three applications per year (each 1 kg ha^{-1}) for a 10 year period under glyphosate selection (3 kg ha^{-1} year^{-1}) for weed control in the fallow period coinciding with the rainy season (November to March). Three seed samples were collected, each consisting of multiple putative glyphosate-resistant *E. colona* individuals. Botanical identification of the plant material was carried out,[22] and seed samples were kept at room temperature.

2.2 Identification and selection of glyphosate-susceptible and glyphosate-resistant *E. colona* individuals from within the field-collected population

In order to identify glyphosate-susceptible and glyphosate-resistant individuals from within the field-collected population, a plant cloning technique was followed.[16,23] This technique enabled the phenotypic identification after glyphosate selection of S plants within a segregating R *E. colona* population (Fig. 1). This approach was conducted outdoors during the normal growing summer season (2011/2012) for *E. colona* in an experimental garden located at the UWA campus (31° 59′ S, 115° 49′ E).

For selection of R plants, seeds were germinated in water solidified agar (0.6% w/v) and transplanted into plastic trays (33.5 × 28 × 6 cm) containing soil. Seedlings at the 2–3-leaf stage were treated with 2160 g ha^{-1} of glyphosate (Roundup PowerMax®; Nufarm, Melbourne, Victoria; 540 g L^{-1}). Plants were maintained outdoors after treatment. Plant survival was recorded 2 weeks after glyphosate treatment, and surviving, growing plants were classified as R plants. Those plants that appeared to be alive without displaying vigorous new growth were unclassified and discarded. For selection of S plants, plants were cloned and numbered. At the 3–4-tiller stage, seedlings were removed from the plastic trays and two tillers per plant (one clone) were excised. These clones were trimmed to 1 cm of shoot material, repotted and numbered accordingly. The ramet plants were transplanted with the same procedures. When the clones achieved the 2–3-leaf stage, they were sprayed with 300 g ha^{-1} of glyphosate. Seedlings that did not survive the glyphosate treatment were classified as S plants. In all cases, glyphosate was applied using a laboratory spray cabinet with a two-nozzle boom delivering a volume of 118 L ha^{-1} of water at a pressure of 210 kPa and travelling at 3.6 km h^{-1} (1 m s^{-1}).

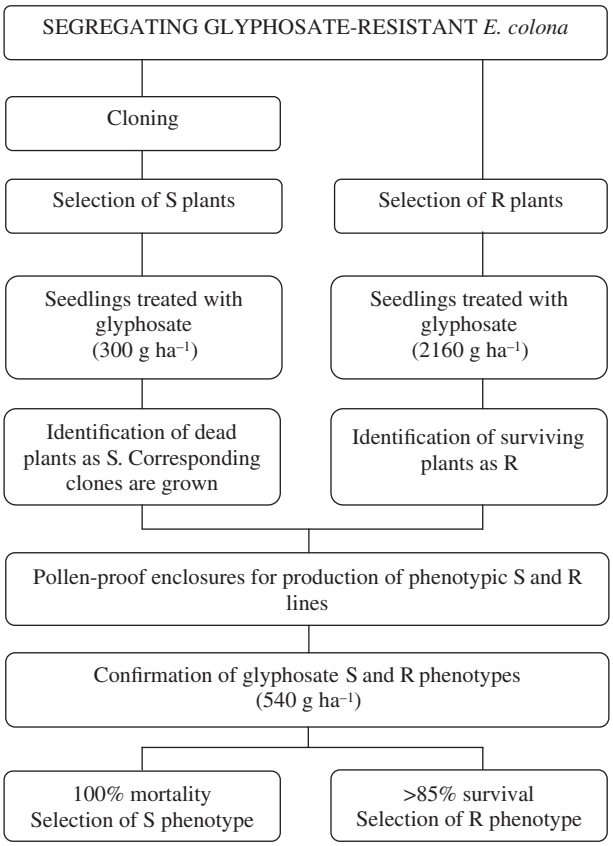

Figure 1. Experimental protocol for the identification and selection of glyphosate-susceptible (S) and glyphosate-resistant (R) *E. colona* phenotypes within a segregating glyphosate-resistant *E. colona* population.

Identified S (selected from the untreated corresponding cloned plants) and R (selected from the treated surviving individuals) plants were individually transferred into bigger pots (24.5 cm in diameter and 27.5 in height) containing a potting mixture (50% composted fine pine bark, 30% cocopeat and 20% river sand). Plants were irrigated as necessary. Pollen-proof enclosures were built to prevent pollen contamination from other sources. No evidence of cross-pollination was observed, even when susceptible and resistant plants were allowed to grow within the same enclosure for experimental purposes. Seeds from individual plants were harvested, cleaned and stored in separate paper bags for further verification.

2.3 Glyphosate resistance profile in the selected glyphosate-susceptible and glyphosate-resistant *E. colona* phenotypes: progeny test

Further glyphosate resistance verification of homogeneous S and R phenotypic lines selected from within the field-collected

E. colona population was conducted after selection with glyphosate at the field recommended dose (540 g ha^{-1}) (Fig. 1).

To break seed dormancy, seeds were pretreated with concentrated sulphuric acid (98%) for 5 min, rinsed with water for 3 min and pregerminated in 500 mL plastic containers containing agar (0.6% w/v) solidified water. Plants were outdoors, with an average air temperature, air relative humidity, light intensity and daylength of 23.5 °C, 43.4%, 856 µmol m^{-2} s^{-1} and 12 h respectively. Seedlings at the 1–2-leaf stage were transplanted into 20-cell plastic trays (33.5 × 28 × 6 cm) that contained a potting mixture (50% composted fine pine bark, 30% cocopeat and 20% river sand) 8 days after germination. A dose of 540 g ha^{-1} of glyphosate was applied to 20 seedlings at the 3–4-leaf stage, collected from 25 individual S and 27 individual R plants. Plant survival was assessed 21 days after glyphosate treatment. This experiment was repeated.

2.4 Phenotypic characterisation of glyphosate resistance: survival, growth and fecundity

Experiments were conducted outdoors during the 2012/2013 summer to characterise the level of glyphosate resistance in the selected R *E. colona* phenotype. For each selected phenotype (bulked seeds after the progeny test in Section 2.3), seeds were germinated as described before. Seed containers were kept outdoors (with a mean light intensity of 640 µmol m^{-2} s^{-1}, an air temperature of 18 °C and an air relative humidity of 71%) for a period of 8 days until the seeds began to germinate.

Twenty germinated seeds of *E. colona* of uniform size at the 2–3-leaf stage from each S and R phenotype were transplanted into 20-cell plastic trays (33.5 × 28 × 6 cm) filled with potting mixture (50% composted fine pine bark, 30% cocopeat and 20% river sand). At the 4–5-leaf stage, seedlings from the S phenotype were treated with glyphosate at doses of 0, 17, 34, 67.5, 135, 270 and 540 g ha^{-1}. Plants from the R phenotype were sprayed with glyphosate at 0, 135, 270, 540, 1080, 2160 and 4320 g ha^{-1}. Seedlings were watered twice a day, and approximately 20 mL of soluble NPKMg fertiliser (19:8.4:15.8:1) (Polyfeed, Haifa Chemicals, Haifa, Israel) was added fortnightly to each cell in a dilute solution (70 g L^{-1}). Trays were arranged in a completely randomised design.

Glyphosate effects on plant survival, above-ground vegetative growth and seed production were determined. Whereas survival (six replicates) and vegetative biomass (three replicates) were assessed 4 weeks after treatment, the seed mass and number were quantified at the end of the growth period (11 weeks after transplanting) (three replicates). Above-ground biomass of surviving plants for each glyphosate dose was harvested, dried at 60 °C for 72 h and weighed.

To minimise the effect of different plant densities on reproductive traits, surviving plants at each glyphosate dose were transplanted individually into pots (24.5 cm diameter and 27.5 cm height) containing a potting mixture (50% composted fine pine bark, 30% cocopeat and 20% river sand). Depending on the survival rate tested on 60 plants, each replicate comprised between three and 15 plants at each glyphosate treatment for both S and R phenotypes. Plants were grown outdoors, watered and fertilised as described above. Seeds were collected from individual plants and kept in paper bags. To minimise seed loss by seed shattering, a PVC-coated fibreglass mesh (approximately 1.4 mm in mesh size) (Cyclone®; Cyclone Industries, Dandenong South, Victoria) was placed under the pots. Seeds were threshed and cleaned by sieving through a series of test sieve mesh sizes (1.5, 1.25 and 1.18 mm) and a fanning mill. Small chaff fragments were manually separated. Total seed mass per plant was determined for each treatment. The weight of 100 seeds was also quantified to estimate the total seed number per plant.

Based on the parameter estimates of the non-linear regression model (see below), the amount of glyphosate to achieve 50% plant mortality (LD_{50}), the vegetative above-ground biomass growth (GR_{50}) and the seed yield (SY_{50}) relative to the untreated control were calculated. Quantitative differences in glyphosate resistance level in terms of either survival, vegetative or reproductive traits between the S and R phenotypes were calculated as a resistance index (RI):

$$RI = \frac{X_{50(R)}}{X_{50(S)}}$$

where X_{50} denotes the LD_{50}, GR_{50} or SY_{50} non-linear regression estimates from the S and R phenotypes.

2.5 Evaluation of other-mode-of-action herbicides

The field-collected *E. colona* population was also subjected to resistance evaluation to herbicides with different modes of action. Similar plant materials and procedures to those described previously were used. Seedlings at the 4–5-leaf stage for both S and R phenotypes were treated with several herbicides of different modes of action (paraquat, glufosinate ammonium, sulfometuron methyl, clethodim, sethoxydim, fluazifop-*p*-butyl, haloxyfop, atrazine and isoxaflutole) at the field recommended dose (see supporting information Table S3).

Responses to pre-emergence herbicides such as trifluralin, pyroxasulfone and *S*-metolachlor were also evaluated. Forty seeds of both S and R phenotypes were scattered onto the soil surface in plastic pots (17.5 cm in diameter and 17.0 cm in height) containing potting mixture prior to herbicide treatments. Seeds were lightly covered with the soil (approximately 0.5 cm depth), watered and left for 2 h to allow the seeds to imbibe water before herbicide treatment. Volatilisation of trifluralin and *S*-metolachlor was minimised by placing a thin layer of soil (approximately 0.5 cm) on the existing potting mixture surface immediately after herbicide treatment.

Survival assessments were conducted in a glasshouse with a mean air temperature of 26 °C. Seedling emergence was recorded 21 days after herbicide treatment (DAT). Each treatment was arranged in a completely randomised design with three replications.

2.6 Statistical analysis

Non-linear regression analysis was carried out to estimate a number of glyphosate resistance parameters (LD_{50}, GR_{50}, SY_{50}) for the S and R *E. colona* phenotypes when exposed to increasing doses of glyphosate. The observed plant survival, vegetative biomass and fecundity data were fitted to an exponential decay model:

$$y = a\, e^{-bx}$$

where y denotes the survival, vegetative biomass or fecundity (mass or number) of plants at glyphosate dose x, a is the maximum plant response and b is the slope (SigmaPlot 12.0 software; Systat Software, Inc., San Jose, CA).

The glyphosate doses resulting in 50% mortality (LD_{50}), growth rate (GR_{50}) and fecundity (SY_{50}) were estimated from the exponential model for both the S and R phenotypes. SY_{50} was predicted

using both total seed mass (SY_{m50}) and total seed number (SY_{n50}) per plant.

Fitness (W) is a function of both the proportion of plants that survive from seed dispersal to reproduction and the amount of offspring produced by adult plants:[24]

$$W = \text{survival rate} \times \text{fecundity}$$

Herbicide selection intensity (SI) for resistance (i.e. selection pressure or effective kill) is a measure of the relative strength of selection for an R phenotype or genotype compared with the S wild-type phenotype. SI can be seen as the relative fitness between R and S phenotypes at the population level under herbicide selection:[14]

$$SI = \frac{W_R}{W_S}$$

where W is the fitness of the phenotype at the population level under a particular herbicide dose.

Fitness and relative selection intensity of the S and R *E. colona* phenotypes were estimated after quantification of both survival and fecundity at two glyphosate doses (540 and 270 g ha^{-1}) using the predicted values from the fitted non-linear regression model, namely the two-parameter exponential decay function $y = a\,e^{-bx}$. The SY and SI values derived from fecundity calculated using seed mass data (see supporting information Fig. S1 and Tables S1 and S2), are similar to those for fecundity estimated from seed number data. Therefore, all results for SI reported herein are from the latter data.

3 RESULTS

3.1 Selection of glyphosate-susceptible and glyphosate-resistant *E. colona* phenotypes

In the first glyphosate bioassay, the clones of 25 plants that did not survive the low glyphosate dose (300 g ha^{-1}) were classified as S plants. Twenty-seven plants survived the high glyphosate dose (2160 g ha^{-1}) and were classified as R plants. Seed was produced.

The selection process in the progeny of the S and R phenotypic lines showed 0 and >85% plant survival, respectively, when exposed to the field recommended glyphosate dose (540 g ha^{-1}). Seeds from their corresponding parent plants (from the first selection) were bulked according to phenotypes and served as the plant materials for the present study. It is noted that all individual plants for both S and R phenotypes show a prostrate growth form.

3.2 Plant survival, growth and fecundity of glyphosate-susceptible and glyphosate-resistant *E. colona* phenotypes under glyphosate selection

Plant survival, vegetative growth and fecundity were significantly different between selected S and R phenotypes under increasing doses of glyphosate (Figs 2 to 4). As expected, the S phenotype was found to be susceptible to glyphosate. The amount of glyphosate required to produce 50% mortality in the R phenotypic line was eightfold greater than that required to control plants of the S phenotypic line. The estimated LD_{50} values for the S and R populations were 173 and 1440 g ha^{-1} respectively (Fig. 2; Table 1). A glyphosate dose of 87 g ha^{-1} caused 50% growth reduction (GR_{50}) in the S phenotype, while the same 50% growth reduction required 693 g ha^{-1} for the R phenotype (Fig. 3; Table 1). Based on these glyphosate doses, the R phenotype was found to be eightfold more resistant to glyphosate than the S phenotype. The significant difference in the LD_{50} and GR_{50} values associated with the

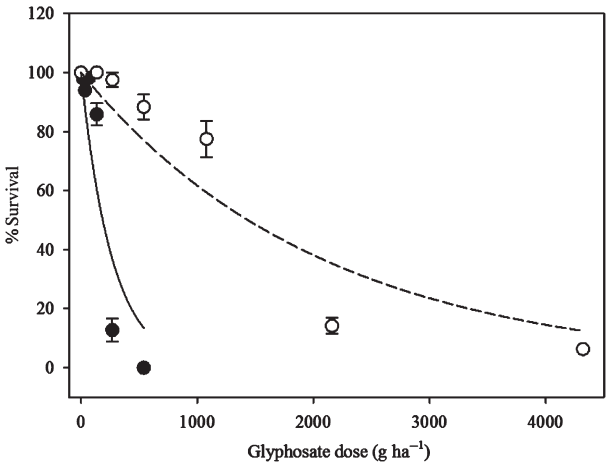

Figure 2. Plant survival as a function of increasing glyphosate doses in glyphosate-susceptible (—●—) and glyphosate-resistant (--○--) *E. colona* phenotypes. Symbols are the mean of six replicates. Symbol bars denote the standard error of the mean. Regression lines represent the fitted exponential decay model.

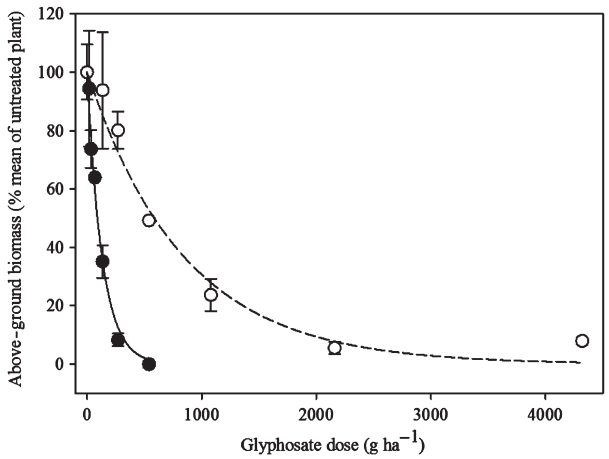

Figure 3. Individual above-ground biomass (% mean of the control) as a function of increasing glyphosate doses in glyphosate-susceptible (—●—) and glyphosate-resistant (--○--) *E. colona* phenotypes. Symbols are the mean of three replicates. Symbols bars denote the standard error of the mean. Regression lines represent the fitted exponential decay model.

R phenotype was due to shoot damage and retarded growth of plants after glyphosate treatment. Estimates of the RI associated with reproductive traits were higher than for survival and vegetative growth. When considering the differences in the glyphosate doses reducing by 50% the individual seed number (SY_{n50}) of the S and R phenotypes (Fig. 4), the RI was 13 (Table 1).

3.3 Assessment of fitness and selection intensity

Plant survival and fecundity (i.e. seed number) of the S and R phenotypes at the recommended glyphosate dose were quantified using the estimated equations from the regression model (Table 2). At the recommended glyphosate dose of 540 g ha^{-1}, the estimated survival rates in the S and R phenotypes were 12 and 77% respectively (Table 2). Compared with plants not treated with glyphosate, as much as 66% of S plant seed production was reduced at the recommended glyphosate dose, whereas for the R phenotype the reduction in individual seed production was 15% (Table 2).

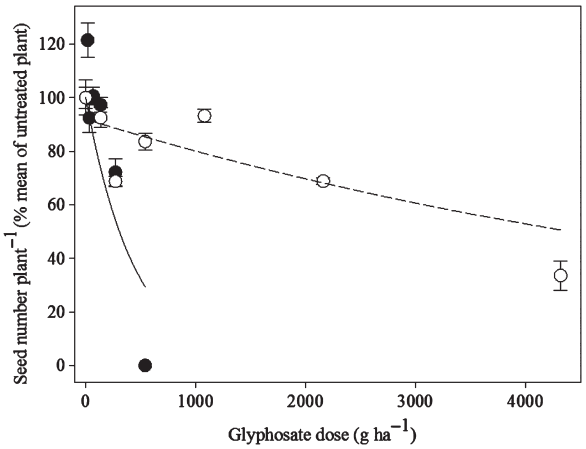

Figure 4. Individual seed number plant (% mean of the control) in glyphosate-susceptible (—●—) and glyphosate-resistant (--○--) *E. colona* phenotypes in response to increasing glyphosate doses. Symbol bars denote the standard error of the mean. Regression lines represent the fitted exponential decay model.

These findings made it possible to estimate the fitness for both the S ($W = 0.04$) and R ($W = 0.66$) phenotypes relative to the fitness under no herbicide treatment ($W = 1$) (Table 2). These results showed that a moderate number of seeds would be produced (34%) by the very few surviving S plants, whereas 85% of the R seeds would be returned to the soil seed bank. Overall, the plants from the R phenotype showed a 17-fold selective advantage compared with plants of the S phenotype when both were exposed to the recommended glyphosate field dose (540 g ha^{-1}).

At a lower glyphosate dose (270 g ha^{-1}), more S and R individuals survived (34 and 88% respectively) (Table 2) than for those plants treated with glyphosate at 540 g ha^{-1}. Seed number production of the S and R phenotypes was reduced by 42 and 11% respectively (Table 2). As a result, the fitness of the S and R individuals when exposed to this glyphosate dose was 20 and 78% of the individual plant fitness in the absence of herbicide treatment. This leads to a fourfold selective advantage for the R plants in comparison with the S plants (SI = 4) (Table 2).

When the glyphosate dose was doubled (from 270 to 540 g ha^{-1}), the fitness of the susceptible plants decreased approximately fivefold (from 0.20 to 0.04) relative to the fitness attained in the absence of selection (1.0) (Table 2). However, the fitness of plants from the R phenotype decreased by a marginal 15%, from 0.78 to 0.66 (Table 2).

3.4 Effect of alternative herbicides

Both S and R plants of *E. colona* were found to be susceptible to all assessed herbicides. At least 60 plants were tested with different herbicide modes of action at normal field doses. There were no survivors of either the glyphosate-susceptible or the glyphosate-resistant *E. colona* when treated with paraquat, glufosinate ammonium, ACCase inhibitors, ALS inhibitors, atrazine, isoxaflutole, trifluralin, pyroxasulfone or *S*-metolachlor (see supporting information Table S3).

4 DISCUSSION

In this study, glyphosate-susceptible and glyphosate-resistant *E. colona* phenotypic lines within a common genetic background were successfully isolated from a glyphosate-resistant population,

Table 1. Estimates of *a*, *b*, LD_{50} (lethal dose), GR_{50} (growth rate) and SY_{50} (seed yield) parameters derived from the exponential decay regression model ($y = a\,e^{-bx}$) for glyphosate-susceptible (S) and glyphosate-resistant (R) *Echinochloa colona* phenotypes[a]

Phenotype	*a*	*b*	R^2	LD_{50}	RI
S	100 (4)	0.0044 (0.0005)	0.83	173	8.3
R	100 (4)	0.0005 (0.00006)	0.86	1440	
Phenotype	*a*	*b*	R^2	GR_{50}	RI
S	100 (6)	0.008 (0.002)	0.83	87	8.0
R	100 (7)	0.001 (0.0002)	0.88	693	
Phenotype	*a*	*b*	R^2	SY_{n50}[b]	RI
S	100 (4)	0.002 (0.0004)	0.62	347	13
R	92 (2)	0.0001 (0.00003)	0.29	4400	

[a] *a* = maximum plant response; *b* = slope of curve; values in parentheses are standard errors of the mean.
[b] $SY_{n50} = SY_{50}$ based on total seed number plant^{-1}.

Table 2. Estimated fitness (*W*) and selection intensity (SI) for glyphosate-susceptible (S) and glyphosate-resistant (R) *Echinochloa colona* phenotypes based on survival rate and fecundity (seed number plant^{-1}) under both glyphosate treatments at 270 and 540 g ha^{-1}

Glyphosate dose (g ha^{-1})	Phenotype	Survival rate[a]	Fecundity (F_n)[b]	Fitness (W)[b]	Glyphosate selection intensity (SI)[b]
270	S	0.34	0.58	0.20	4
	R	0.88	0.89	0.78	
540	S	0.12	0.34	0.04	17
	R	0.77	0.85	0.66	

[a] Glyphosate selection intensity (SI) based on plant survival at 540 g ha^{-1} = 0.77/0.12 = 6.
[b] F_n = fecundity based on total seed number plant^{-1}; W = survival rate × fecundity; $SI = W_R/W_S$.

and the level of glyphosate resistance was assessed by quantification of plant survival, vegetative growth and fecundity responses to increasing glyphosate doses. Plant fitness and selection intensity for glyphosate resistance were evaluated to provide insight into the rate of glyphosate resistance evolution in the studied *E. colona* population.

4.1 Glyphosate resistance level (resistance index)

The results provide evidence that the *E. colona* population originating from the northern region of Western Australia has evolved resistance exclusively to glyphosate. There was no resistance across a range of herbicides of different modes of action. Based on plant survival and vegetative growth responses to a wide range of glyphosate doses, an eightfold glyphosate resistance was found. This glyphosate resistance index is consistent with a previous report on an *E. colona* population from the same agricultural region.[19] The biochemical basis of glyphosate resistance in this *E. colona* phenotype is under investigation.

In this study, when considering the seed number produced by individuals from the S and R phenotypes under the effect of increasing glyphosate doses, the resistance index was higher (RI = 13) compared with the resistance level based on estimations of plant survival rate. This shows that resistance differences between the S and R phenotypes are quantitatively larger for resource allocation to reproduction than the ability to survive the glyphosate treatment.

Thus, the quantification of herbicide resistance should also consider an ecological measure that quantifies the allelic contribution for the next generations of S and R phenotypes under the selection of herbicide field doses. Plant fitness attained in this environment is a major ecological trait that assimilates not only the proportion of plants that survive a herbicide treatment but also their fecundity as contributions to the next generation.[25]

4.2 Fitness and selection intensity for glyphosate resistance

Plant fitness values of both S and R phenotypes were markedly different under low versus high glyphosate doses. From a weed management viewpoint, this indicates the importance of using the recommended glyphosate field doses to avoid a rapid increase in plant densities in the next generations. Low herbicide doses increase the competitive weed–crop interactions, as larger weed populations will persist in the environment with eventual reductions in crop yields. Furthermore, previous studies have demonstrated that the use of herbicide doses below the recommended field doses often leads to rapid herbicide resistance evolution by the accumulation and selection of minor resistance gene traits within treated weed populations.[26–29]

Quantification of the selection intensity for resistance (i.e. relative R:S fitness under glyphosate selection) is an important parameter that helps to predict the dynamics of glyphosate resistance alleles in agroecosystems. Empirical estimations of herbicide selection intensities are lacking in the literature.[13,14] Herbicide selection intensity can be seen as the rate of relative enrichment in the environment of R plants in relation to S plants. The present study reports that under 540 g ha^{-1} of glyphosate there is a 17-fold selective advantage for plants carrying the glyphosate resistance trait compared with plants with the susceptible trait. This study also highlights that any empirical attempt to estimate herbicide selection intensities based on plant survival assessments are not accurate. For example, we would have underestimated the intensity of selection and enrichment of glyphosate R alleles (SI = 6) (Table 2). However, it is important to highlight that an even more accurate estimation of the glyphosate selection intensity would be in R and S plants in competition with a crop.

Acknowledgement of the simple evolutionary ecology context for glyphosate resistance used in the present study requires the correct inclusion and empirical assessment of plant fitness, an issue that has often been overlooked.

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SUPPORTING INFORMATION

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

REFERENCES

1 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**(6):489–499 (2012).
2 Jasieniuk M, Brûlé-Babel AL and Morrison IN, The evolution and genetics of herbicide resistance in weeds. *Weed Sci* **44**:176–193 (1996).
3 Preston C and Powles S, Evolution of herbicide resistance in weeds: initial frequency of target site-based resistance to acetolactate synthase-inhibiting herbicides in *Lolium rigidum*. *Heredity* **88**(1):8–13 (2002).
4 Powles SB and Yu Q, Evolution in action: plants resistant to herbicides. *Annu Rev Plant Biol* **61**:317–347 (2010).
5 Binkholder KM, Fresenburg BS, Teuton TC, Xiong X and Smeda RJ, Selection of glyphosate-resistant annual bluegrass (*Poa annua*) on a golf course. *Weed Sci* **59**:286–289 (2011).
6 Powles SB and Howat PD, Herbicide-resistant weeds in Australia. *Weed Technol* **4**(1):178–185 (1990).
7 Powles SB, Lorraine-Colwill DF, Dellow JJ and Preston C, Evolved resistance to glyphosate in rigid ryegrass (*Lolium rigidum*) in Australia. *Weed Sci* **46**(5):604–607 (1998).
8 Maxwell BD and Mortimer A, Selection for herbicide resistance, in *Herbicide Resistance in Plants: Biology and Biochemistry*, ed. by Powles SB and Holtum JAM. Lewis, Boca Raton, FL, pp. 1–25 (1994).
9 Purba E, Preston C and Powles SB, The mechanism of resistance to paraquat is strongly temperature dependent in resistant *Hordeum leporinum* Link and *H. glaucum* Steud. *Planta* **196**(3):464–468 (1995).
10 Vila-Aiub MM, Gundel PE, Yu Q and Powles SB, Glyphosate resistance in *Sorghum halepense* and *Lolium rigidum* is reduced at suboptimal growing temperatures. *Pest Manag Sci* **69**(2):228–232 (2013).
11 Beckie HJ, Heap IM, Smeda RJ and Hall LM, Screening for herbicide resistance in weeds. *Weed Technol* **14**(2):428–445 (2000).
12 Burgos NR, Tranel PJ, Streibig JC, Davis VM, Shaner D, Norsworthy JK *et al.*, Review: confirmation of resistance to herbicides and evaluation of resistance levels. *Weed Sci* **61**(1):4–20 (2013).
13 Beckie HJ and Morrison IN, Effect of ethalfluralin and other herbicides on trifluralin-resistant green foxtail (*Setaria viridis*). *Weed Technol* **7**(1):6–14 (1993).
14 Beckie HJ and Morrison IN, Effective kill of trifluralin-susceptible and -resistant green foxtail (*Setaria viridis*). *Weed Technol* **7**(1):15–22 (1993).
15 Vila-Aiub MM, Neve P and Powles SB, Fitness costs associated with evolved herbicide resistance alleles in plants. *New Phytol* **184**(4):751–767 (2009).
16 Vila-Aiub MM, Neve P and Roux F, A unified approach to the estimation and interpretation of resistance costs in plants. *Heredity* **107**(5):386–394 (2011).
17 Heap I, *The International Survey of Herbicide Resistant Weeds*. [Online]. Available: www.weedscience.com [23 January 2015].
18 Alarcón-Reverte R, García A, Urzúa J and Fischer AJ, Resistance to glyphosate in junglerice (*Echinochloa colona*) from California. *Weed Sci* **61**(1):48–54 (2013).

19 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).
20 Neve P, Simulation modelling to understand the evolution and management of glyphosate resistance in weeds. *Pest Manag Sci* **64**(4):392–401 (2008).
21 Thornby DF and Walker SR, Simulating the evolution of glyphosate resistance in grains farming in northern Australia. *Ann Bot* **104**(4):747–756 (2009).
22 Tabacchi M, Mantegazza R, Spada A and Ferrero A, Morphological traits and molecular markers for classification of *Echinochloa* species from Italian rice fields. *Weed Sci* **54**(6):1086–1093 (2006).
23 Vila-Aiub MM, Neve P, Steadman KJ and Powles SB, Ecological fitness of a multiple herbicide-resistant *Lolium rigidum* population: dynamics of seed germination and seedling emergence of resistant and susceptible phenotypes. *J Appl Ecol* **42**(2):288–298 (2005).
24 Futuyma DJ, *Evolution*. Sinauer Association, Inc., Sunderland, MA (2013).
25 Neve P, Busi R, Renton M and Vila-Aiub MM, Expanding the eco-evolutionary context of herbicide resistance research. *Pest Manag Sci* **70**(9):1385–1393 (2014).
26 Busi R and Powles SB, Evolution of glyphosate resistance in a *Lolium rigidum* population by glyphosate selection at sublethal doses. *Heredity* **103**(4):318–325 (2009).
27 Manalil S, Busi R, Renton M and Powles SB, Rapid evolution of herbicide resistance by low herbicide dosages. *Weed Sci* **59**(2):210–217 (2011).
28 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**(6):1154–1166 (2005).
29 Neve P, Vila-Aiub M and Roux F, Evolutionary-thinking in agricultural weed management. *New Phytol* **184**(4):783–793 (2009).

