Glyphosate resistance in *Echinochloa colona*: phenotypic characterization 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 (susceptibility '*S*' *vs* resistance '*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 characterize 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) have shown a 8-fold glyphosate resistance in this population. The resistant index based on the estimation of seed number (SY_{n50}) shows a 13-fold resistance. As a result of linear combination of plant survival and fecundity rates, plant fitness of 0.2 and 0.8 was 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 5-fold 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 the 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.

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 where 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 (gene mutation rate, dominance, additivity, epistasis, pleiotrophy, inheritance mode, ploidy), biological (reproduction and mating system, population size, number of generations) factors[2,8] and 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 inform on 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 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. Despite its importance, very few studies have empirically estimated the intensity of herbicide selection (*SI*)[13,14]. For example, Beckie and Morrison estimated a 29-fold selective advantage of trifluralin-resistant compared to trifluralin-susceptible plants treated at the full recommended dose[14]**.** 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 USA[18,19].

In this study we characterize 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 (WA). 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 WA, 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 (*S*) and -resistant (*R*) *E. colona* individuals from within the field collected population

In order to identify glyphosate-susceptible and -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 susceptible (*S*) plants within a segregating glyphosate-resistant (*R*) *E. colona* population (Fig. 2.1). 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 (S 31°59'; E115°49').

For selection of *R* plants, seeds were germinated in water solidified agar (0.6% w/v) and transplanted into plastic trays (33.5 x 28 x 6 cm) containing soil. Seedlings at the 2-3-leaf stage were treated with 2,160 g ha^{-1} of glyphosate (Roundup PowerMax®, Nufarm, Australia; 540 g L^{-1}). Plants were maintained outdoors after treatment. Plant survival was recorded two 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 two- to three-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 2-nozzle boom delivering a volume of 118 L ha^{-1} water at a pressure of 210 kPa travelling at 3.6 km h^{-1} (1 m s^{-1}).

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 (*S*) and -resistant (*R*) *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. 2.1).

To break seed dormancy seeds were pre-treated with concentrated sulphuric acid (98%) for five minutes and rinsed with water for three minutes and pre-germinated in 500 ml plastic containers containing agar (0.8%) solidified water. Plants were outdoors with an average of 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 x 28 x 6 cm) which contained a potting mixture (50% composted fine pine bark, 30% cocopeat and 20% river sand) eight days after germination. A dose of 540 g ha^{-1} of glyphosate was applied to 20 seedlings at 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 characterization of glyphosate resistance: survival, growth and fecundity

Experiments were conducted outdoors during the 2012/2013 summer to characterize 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 mean light intensity of 640 μmol m^{-2} s^{-1}, air temperature of 18°C and air relative humidity of 71%) for a period of eight days until the seeds begin to germinate.

Twenty germinated seeds of *E. colona* with uniform size at the 2-3 leaf stage from each *S* and *R* phenotypes were transplanted into 20-cell plastic trays (33.5 x 28 x 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 the following doses: 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 fertilizer (19:8.4:15.8:1) (Poly-feed, Israel) were added fortnightly to each cell in a dilute solution (70 g L^{-1}). Trays were arranged in a completely randomized design.

Glyphosate effects on plant survival, aboveground vegetative growth and seed production were determined. Whereas survival (6 replicates) and vegetative biomass (3 replicates) was assessed 4 weeks after treatment, the seed mass and number was quantified at the end of the growth period (11 weeks after transplanting) (3 replicates). Aboveground biomass of surviving plants for each glyphosate dose was harvested, dried at 60°C for 72 hours and weighed.

To minimize 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 fifteen 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 minimize seed loss by seed shattering, a PVC coated fiberglass mesh (approximately 1.4 mm in mesh size) (Cyclone®, Australia) 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 fanning mill. Small chaff fragments were manually separated. Total seed mass per plant was determined for each treatment. 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}), vegetative aboveground biomass growth (GR_{50}) and seed yield (SY_{50}) relative to the untreated control was 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 glyphosate-susceptible (*S*) and -resistant (*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 were used as described previously. Seedlings at 4-5 leaf stage for both *S* and *R* phenotypes were treated with several different herbicides of different modes of action (paraquat, glufosinate ammonium, sulfometuron methyl, clethodim, sethoxydim, fluazifop-p-butyl, haloxyfop, atrazine and isoxaflutole) at field recommended dose (Supporting Information Table S3).

Response 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 (approx. 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 (approx. 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 treatments (DAT). Each treatment was arranged in complete 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:

$$y = ae^{-bx}$$

where *y* denotes 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, CA, USA).

The glyphosate dose reducing 50% mortality (LD_{50}), growth rate (GR_{50}) and fecundity (SY_{50}) was 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) and selection intensity (SI)

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]:

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

Herbicide selection intensity (*SI*) for resistance (i.e. selection pressure or effective kill) is a measure of the relative strength of selection for a resistant (*R*) phenotype or

genotype compared to the susceptible (*S*) wild type phenotype. Selection intensity (*SI*) can be seen as the relative fitness between a resistant (*R*) and susceptible (*S*) phenotype at the population level under herbicide selection[14]:

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

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

Fitness and relative selection intensity (*SI*) 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, viz. two-parameter exponential decay function ($y = ae^{-bx}$). The *SY* and *SI* values that derived from fecundity, which calculated using seed mass data (See Supporting Information, Fig. S1, Table S1 and S2), are similar to those for fecundity that 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 (*S*) and -resistant (*R*) *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 a 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 (*S*) and -resistant (*R*) *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. 2.2, 2.3, 2.4). 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 8-fold greater than that required to control plants of the *S* phenotypic line. The estimated LD_{50} values for the *S* and *R* population were 173 and 1440 g ha^{-1}, respectively (Fig. 2.2; Table 2.1). A glyphosate dose of 87 g ha^{-1} caused 50% growth reduction (GR_{50}) in the *S* phenotype meanwhile the same 50% growth reduction required 693 g ha^{-1} for the *R* phenotype (Fig. 2.3; Table 2.1). Based on these glyphosate doses, the *R* phenotype was found to be 8-fold more resistant to glyphosate, relative to the *S* phenotype. The significant difference in the LD_{50} and GR_{50} values associated with the *R* phenotype was due to shoot damage and retarded growth of plants after glyphosate treatment. Estimates of the resistance index (*RI*) associated with reproductive traits were higher than for survival and vegetative growth. When considered the differences in the glyphosate doses to reduce 50% the individual seed number (SY_{n50}) of the *S* and *R* phenotypes (Figs. 2.4), the *RI* was 13 (Table 2.1).

3.3 Assessment of fitness (*W*) and selection intensity (*SI*)

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 2.2). At the recommended glyphosate dose of 540 g ha^{-1}, the estimated survival rate in the *S* and *R* phenotypes was 12% and 77%, respectively (Table 2.2). Compared to glyphosate untreated plants, as much as 66% of *S* plant seed production was reduced at the recommended glyphosate dose whereas for the *R* phenotype, reduction in individual seed production was 15% (Table 2.2).

These findings enabled the estimation of 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 2.2). These results showed that a moderate number of seeds will be produced (34%) by the very few surviving *S* plants whereas 85% of the *R* seeds will be returned to the soil seed bank. Overall, the plants from the *R* phenotype show a 17-fold selective advantage compared to 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 2.2) than 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 2.2). As a result, fitness of the *S* and *R* individuals when exposed to this glyphosate dose was 20% and 78% of the individual plant fitness in absence of herbicide treatment. This leads to a 4-fold selective advantage for the *R* plants in comparison to S plants (SI = 4; Table 2.2).

When doubling the glyphosate dose (from 270 to 540 g ha^{-1}), the fitness of the susceptible plants decreased approximately 5-fold (from 0.20 to 0.04) (relative to the fitness attained in absence of selection, 1.0) (Table 2.2). However, the fitness of plants from the *R* phenotype decreased a marginal 15%, from 0.78 to 0.66 (Table 2.2).

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 *S* or *R 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 -resistant *E. colona* phenotypic lines within a common genetic background were successfully isolated from a glyphosate-resistant population and the level of glyphosate resistance 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.

Glyphosate resistance level (resistance index)

The results provide evidence that the *E. colona* population which originated from the northern region of WA has evolved resistance exclusively to glyphosate. There was no resistance across a range of herbicide of different modes of action. Based on plant survival and vegetative growth responses to a wide range of glyphosate doses, a 8-fold glyphosate resistance was found. This glyphosate resistance index is consistent with a previous report on

a *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 to 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 next generations of *S* and *R* phenotypes under the selection of the herbicide field doses. Plant fitness attained under 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 contribution to the next generation[25].

Fitness and selection intensity for glyphosate resistance

Plant fitness of both *S* and *R* phenotypes were markedly different under low *vs* high glyphosate doses. From a weed management view, this notes the importance of using the recommended glyphosate field doses to avoid a rapid increase of 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 use of herbicide doses below the recommended field ones often lead 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 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 to 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 2.2). However, it is important to highlight that an even more accurate estimation of the glyphosate selection intensity would be in *R* and *S* plants under competition with a crop.

Acknowledgment of this 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 been often overlooked.

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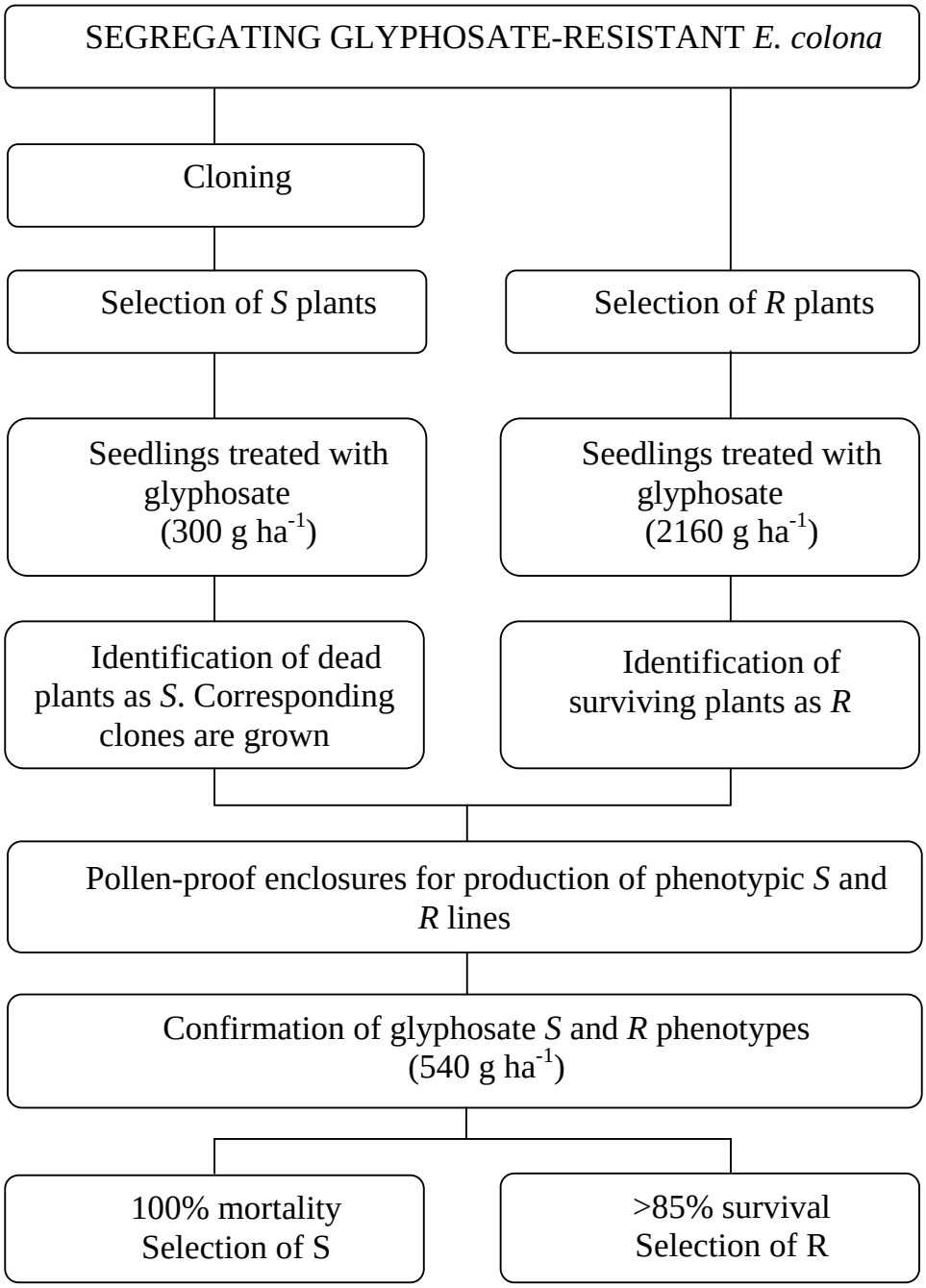

Figure 2.1 Experimental protocol for the identification and selection of glyphosate-susceptible (*S*) and -resistant (*R*) *E. colona* phenotypes within a segregating glyphosate resistant *E. colona* population.

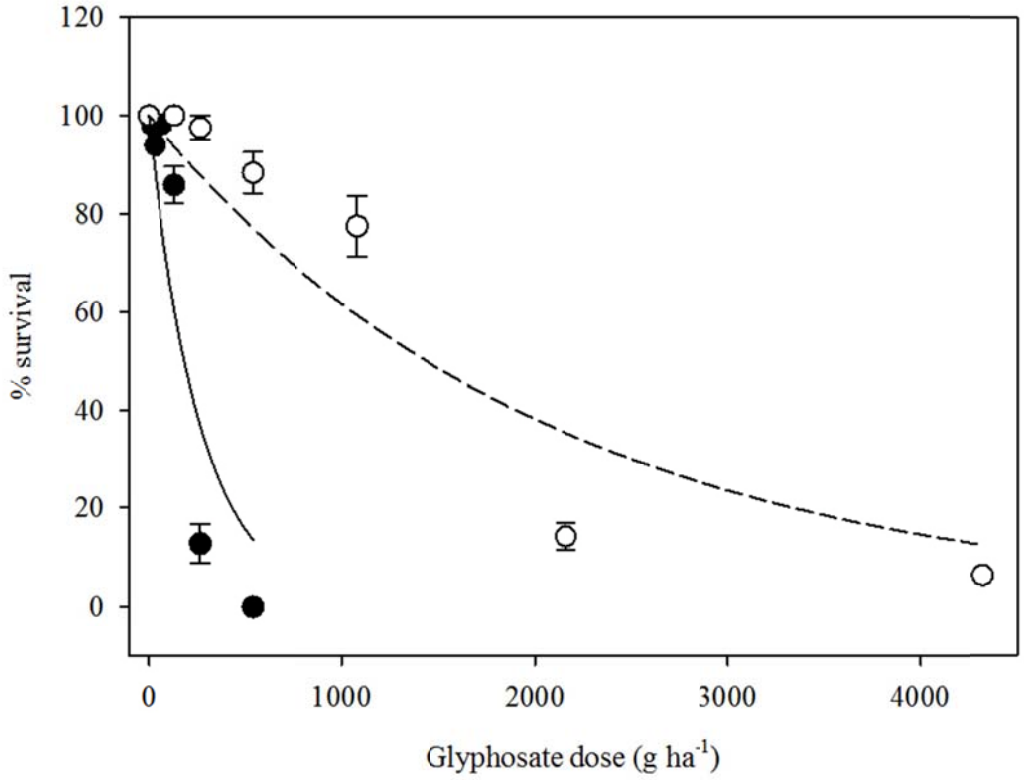

Figure 2.2 Plant survival as a function of increasing glyphosate doses in glyphosate-susceptible (*S*; ●) and -resistant (*R*; ○) *E. colona* phenotypes. Symbols are mean of six replicates. Symbol bars denote the standard error of the mean. Regression lines represent the fitted exponential decay model.

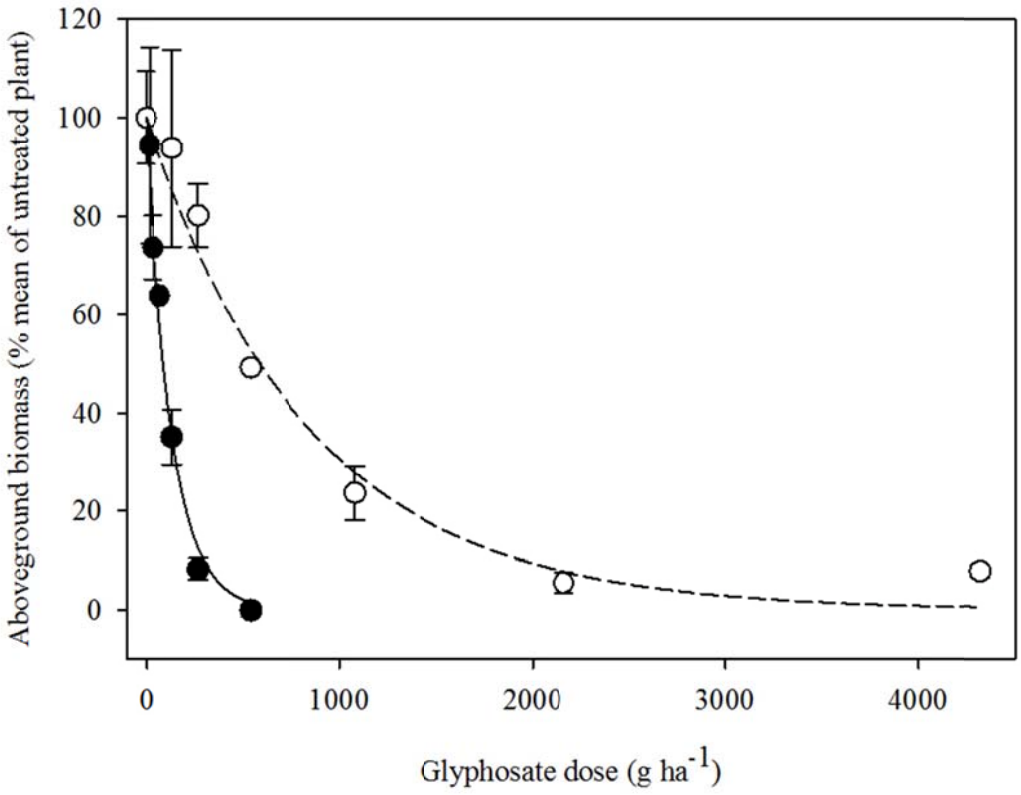

Figure 2.3 Individual aboveground biomass (% mean of the control) as a function of increasing glyphosate doses in glyphosate-susceptible (*S*; •) and -resistant (*R*; o) *E. colona* phenotypes. Symbols are mean of three replicates. Symbols bars denote the standard error of the mean. Regression lines represent the fitted exponential decay model.

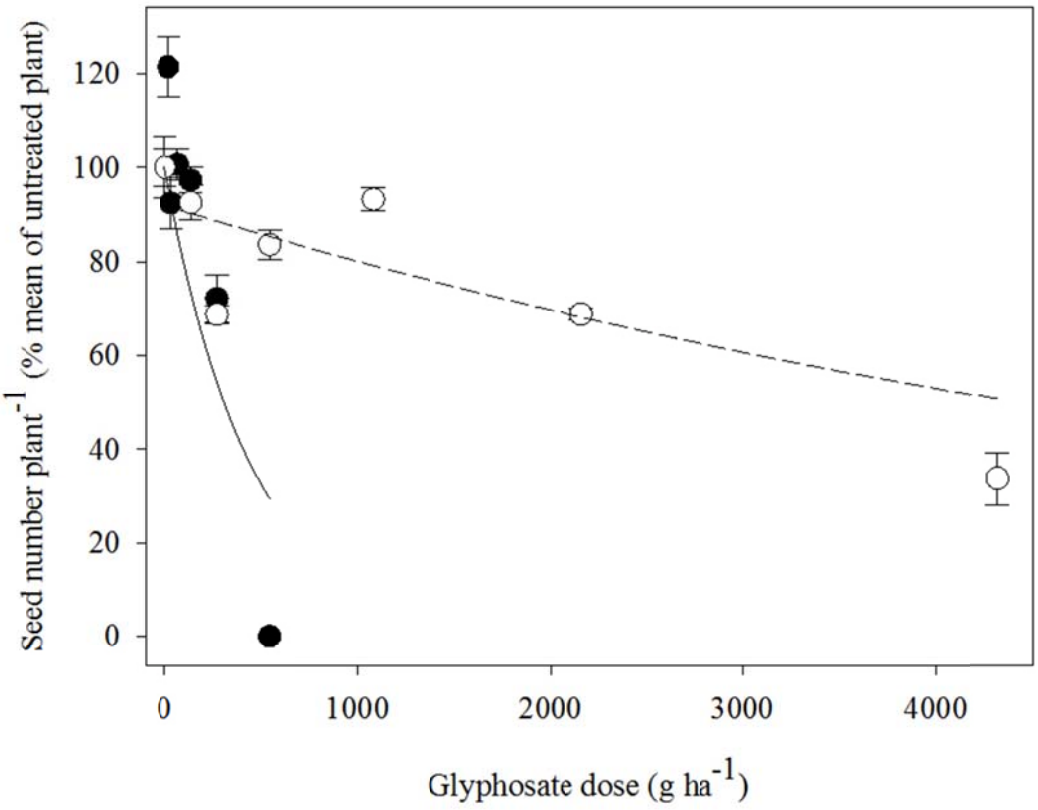

Figure 2.4 Individual seed number plant (% mean of the control) in glyphosate-susceptible (*S*; •) and -resistant (*R*; o) *E. colona* phenotypes in response to increasing glyphosate doses. Symbol bars denote the standard error of the mean. Regression lines represent the fitted exponential decay model.

Table 2.1 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 ($y = ae^{-bx}$) for glyphosate-susceptible (*S*) and -resistant (*R*) *Echinochloa colona* phenotypes[1]

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	
	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	
	a	***b***	R^2	SY_{n50}^{+}	***RI***
S	100 (4)	0.002 (0.0004)	0.62	347	13
R	92 (2)	0.0001 (0.00003)	0.29	4400	

[1] *a* = the maximum plant response, *b* = the slope of curve. Values in parenthesis are standard errors of the mean

[+] SY_{n50}: SY_{50} based on total seed number plant^{-1}

Table 2.2 Estimated fitness (*W*) and selection intensity (*SI*) for glyphosate-susceptible (*S*) and -resistant (*R*) *Echinochloa colona* phenotypes based on survival rate and fecundity (seed number plant^{-1}) under both glyphosate treatment at 270 and 540 g ha^{-1}

Glyphosate dose (g ha^{-1})	**Phenotype**	**Survival rate[#]**	**Fecundity (F_n)[+]**	**Fitness (W)[+]**	**Glyphosate selection intensity (SI)[+]**
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	

[+] F_n = fecundity based on total seed number plant^{-1}, W = survival rate x fecundity, SI = $\frac{W_R}{W_S}$

[#] Glyphosate selection intensity (*SI*) based on plant survival at 540 g ha^{-1} = $\frac{0.77}{0.12}$ = 6