1 **Multiple resistance across glufosinate, glyphosate, paraquat and ACCase-inhibiting**

2 **herbicides in an *Eleusine indica* population**

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13 **Running head:** Multiple resistance in *Eleusine indica*

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20   **Summary**

21   An *Eleusine indica* population was previously reported as the first global case of field-

22   evolved glufosinate resistance. This study re-examines glufosinate resistance and investigates

23   multiple resistance to other herbicides in the population. Dose-response experiments with

24   glufosinate showed that the resistant population is 5-fold and 14-fold resistant relative to the

25   susceptible population, based on GR_{50} and LD_{50} R/S ratio, respectively. The selected

26   glufosinate-resistant sub-population also displayed a high level resistance to glyphosate, with

27   the respective GR_{50} and LD_{50} R/S ratios being 12- and 144-fold. In addition, the sub-

28   population also displayed a level of resistance to paraquat and ACCase-inhibiting herbicides

29   fluazifop-P-butyl, haloxyfop-P-methyl and butroxydim. ACCase gene sequencing revealed

30   that the Trp-2027-Cys mutation is likely responsible for resistance to the ACCase inhibitors

31   examined. Here we confirm glufosinate resistance and importantly, we find very high level

32   glyphosate resistance, as well as resistance to paraquat and ACCase inhibiting herbicides.

33   This is the first confirmed report of a weed species that evolved multiple resistance across all

34   the three non-selective global herbicides, glufosinate, glyphosate and paraquat.

35   **Keywords**: Herbicide resistance, Indian goosegrass**,** non-selective herbicides, fluazifop-P-

36   butyl

Introduction

Eleusine indica (L.) Gaertn. (Indian goosegrass), one of the world's worst weeds (Holm *et al*., 1977), is a very competitive and cosmopolitan species. *Eleusine indica* is fecund, found across a range of soils and temperatures (Nishimoto & McCarty, 1997) and infests a wide range of crops including cotton, maize, upland rice, sweet potatoes, sugarcane and many fruit and vegetable orchards (Holm *et al*., 1977), causing major crop yield loss (Lourens *et al*., 1989).

In tropical countries such as Malaysia, *E. indica* infestation occurs mostly in field crops areas, fruit and vegetable orchards, nurseries and young palm oil plantations. *Eleusine indica* has been shown to affect crop growth, cause yield loss and increase the incidence of plant disease such as *Phytophtora* spp. (Chee *et al*., 1990; Teng & Teo, 1999). Control of *E. indica* is mainly with herbicides, but over reliance on herbicides has resulted in resistance evolution in this species in at least eight countries (Heap, 2013). This includes resistance to dinitroaniline herbicides (Mudge *et al*., 1984), acetyl coA carboxylase (ACCase)-inhibiting herbicides (Leach *et al*., 1993; Osuna *et al*., 2012), the acetolactate synthase (ALS)-inhibiting herbicide imazapyr (Valverde *et al*., 1993), the glycine herbicide glyphosate (Lee & Ngim, 2000), the bipyridilium herbicide paraquat (Buker *et al*., 2002), photosystem II inhibitors (Brosnan *et al*., 2008) and, most recently, the glutamine synthetase-inhibiting herbicide glufosinate (Jalaludin *et al*., 2010; Chuah *et al*., 2010).

Glyphosate, and its alternative, glufosinate, are two of the most important herbicides globally. Glyphosate was initially used in Malaysia to control *E. indica* and other weeds in fallows, nurseries and to remove ground cover vegetation in plantations. Over-reliance on glyphosate was a strong selection pressure and glyphosate resistance in *E. indica* quickly evolved (Lee & Ngim, 2000). Now, many *E. indica* populations have been identified as

61 glyphosate resistant (Ng *et al*., 2003; Ng *et al*., 2004; Kaundun *et al*., 2008). In response to

62 glyphosate-resistant evolution in *E. indica*, high glufosinate usage has occurred. In 2010, the

63 first case of glufosinate resistance was reported in a Malaysia *E. indica* population (Jalaludin

64 *et al*., 2010). Prior to glufosinate usage, this resistant population had a field history of

65 paraquat, fluazifop-P-butyl and glyphosate treatment.

66   At the same time, another Malaysian *E. indica* population was reported to be resistant

67 to glufosinate and paraquat (Chuah *et al*., 2010). Subsequently, glufosinate resistance and

68 multiple-resistance to glufosinate and glyphosate have been reported in *Lolium perenne* L.

69 populations in Oregon, USA (Avila-Garcia & Mallory-Smith, 2011; Avila-Garcia *et al*.,

70 2012). The objective of this study was to characterise the glufosinate resistant population

71 from the preliminary study by Jalaludin *et al.*(2010) and evaluate for possible multiple

72 resistance to herbicides of different modes of action.

73 **Materials and methods**

74 *Plant material*

75 The glufosinate resistant (R) *E. indica* population used in this study was preliminarily

76 described (Jalaludin *et al*., 2010). A glufosinate-susceptible population was originally

77 provided by T S Chuah and a subset of this population that was confirmed to be susceptible

78 to all herbicides examined in the current study was generated and used as the herbicide

79 susceptible (S) population.

80 *Glufosinate dose response*

81 *Eleusine indica* seeds were germinated on water-solidified 0.6% agar containing 0.2%

82 potassium nitrate (KNO_3) (Ismail *et al*., 2002). After 4-7 days, seedlings were transplanted

83 into pots (18 cm diameter with 15-20 seedlings per pot) and kept in a glasshouse during the

84 normal summer growing months (January to March) with average temperatures of 30/20°C

85 (day/ night), and 15 hours photoperiod under natural sunlight. At the 3-5 leaf stage, seedlings

86 were treated at various rates of glufosinate (0, 20.6, 41.3, 82.5, 123.8, 247.5, 495, 1485, 1980,

87 3960 and 7920 g a.i. ha^{-1}) (Basta, 200 g a.i. L^{-1}, SC, Bayer CropScience Pty Ltd), using a

88 custom-built, dual nozzle cabinet sprayer delivering herbicide at 118 L ha^{-1} at 210 kPa, with a

89 speed of 1 m s^{-1}. After herbicide treatment, plants were returned to the glasshouse. The pots

90 were arranged in a completely randomised block design with at least three replicate pots per

91 herbicide rate. Visual assessment for resistance (R) and susceptibility (S) were made 21 days

92 after treatment. Plants were considered as R if they are actively growing or tillering, while S

93 plants were dead. Above-ground shoots were harvested and dried in oven (65°C) for 3 days

94 for dry weight measurements.

95   Additionally, six individual plants surviving 1485 and 1980 g a.i.ha^{-1} of glufosinate

96 were allowed to grow together to produce seeds (*E. indica* is a self-pollinated species) and the

97 progeny was designated as selected glufosinate-resistant sub-population (referred as R*).

98 This sub-population was tested again for glufosinate resistance and used for subsequent

99 experiments.

100 *Glyphosate dose response*

101 Seed germination and seedling growth were the same as described above for glufosinate

102 experiments. Glyphosate rates at 0, 33.8, 67.5, 100, 135, 170, 200, 540, 1080, 4320, 8640,

103 12960, 17280 and 25920 g a.e. ha^{-1} (Roundup Attack with IQ inside, 570 g a.e. L^{-1}, SL,

104 Nufarm Australia Ltd) were used.

105 *Paraquat dose-response*

Seed germination was carried out as described earlier. After transplanting into pots, the seedlings were grown in a controlled environment room with alternating temperatures of 30/25°C (day/ night), 12 hours photoperiod with light intensity of 400 μmol m^{-2} s^{-1} and 75% humidity. At the 3-4 leaf stage, the plants were treated with paraquat at 0, 47, 94, 188, 375, 750, 1500 and 3000 g a.i. ha^{-1} (Gramoxone, 250 g a.i. L^{-1}, SL, Syngenta Crop Protection Pty Ltd).

Herbicide single-rate test

In this experiment, germinating seedlings were transplanted to trays (50-60 seedlings per tray with two to four trays per herbicide treatment) and kept in a glasshouse with day/night temperature of 30/25°C under natural sunlight. Single discriminating or label rates of ACCase-inhibiting herbicides fluazifop-P-butyl, 210 g a.i. ha^{-1} (Fusilade Forte, 128 g a.i. L^{-1}, EC, Syngenta Crop Protection Pty Ltd), haloxyfop-P-methyl, 60 g a.i. ha^{-1} (Verdict 520, 520 g a.i. L^{-1}, EC, Dow Agrosciences Australia Ltd), clethodim, 100 g a.i. ha^{-1} (Select, 240 g a.i. L^{-1}, EC, Sumitomo Chemical Australia Pty Ltd), butroxydim, 100 g a.i. ha^{-1} (Falcon, 250 g a.i. kg^{-1}, WG, Nufarm Australia Ltd) and sethoxydim, 230 g a.i. ha^{-1} (Sertin 186 EC, 186 g a.i. L^{-1}, EC, Bayer CropScience Pty Ltd), and the ALS-inhibiting herbicide imazapyr, 50 g a.i. ha^{-1} (Arsenal, 250 g a.i. L^{-1}, SC, Nufarm Australia Ltd) was used for resistance screening.

Statistics

The herbicide rate causing 50% mortality (LD_{50}) or reduction in growth (GR_{50}) were estimated by non-linear regression analysis using Sigma Plot ® software (version 12.0, SPSS Inc. 233 South Wacker Drive, Chicago, IL, USA). The data were fitted to the three parameter logistic curve model:

129 $$y = \frac{a}{1+(\frac{x}{ED_{50}})^b}$$ Eq. 1

130

131 where a = upper limit, ED_{50} = estimated dose causing 50% response (LD_{50} or GR_{50}) and b =

132 slope around ED_{50}. The LD_{50} and GR_{50} values of the susceptible and resistant biotypes were

133 used to calculate the R/S ratio of the resistant population. There were several pilot trials prior

134 to final herbicide dose response experiments, which contained at least three replicate pots per

135 herbicide rate. Each dose-response experiment was repeated at least twice with similar results

136 and therefore only results from a single experiment were presented for each dose response.

137 *ACCase gene sequencing*

138 Genomic DNA was extracted from the leaf tissue of surviving plants from R* population and

139 susceptible plants from S population according to Yu *et al*. (2008). Published primers (Osuna

140 *et al*., 2012) used to amplify two plastidic ACCase gene fragments in which point mutations

141 known to confer ACCase herbicide resistance in plants have been identified (Délye &

142 Michel, 2005; Powles & Yu, 2010; Beckie & Tardif, 2012). The PCR was conducted in a 25

143 µl volume that consisted of 1-2 µl containing 50-100 ng of genomic DNA, 0.5 µM of each

144 primer and 12.5 µl of 2× GoTaq Green Master Mix® (Promega). The PCR was run with the

145 following profile: $94^{o}C$ for 4 min; 40 cycles of $94^{o}C$ for 30 s, $58^{o}C$ (annealing temperature)

146 for 30 s, and $72^{o}C$ for 1 min; followed by a final extension step of 7 min at $72°C$. The PCR

147 product was purified from agarose gel with Wizard[®] SV Gel and PCR Clean-up System

148 (Promega Co., Madison, WI, USA) and sequenced by commercial services. All sequence

149 chromatograms were visually checked for quality and consistency before sequences were

150 assembled and aligned.

151 **Results**

Glufosinate resistance

153   As expected, the S plants were well controlled with glufosinate (Fig. 1). In contrast, much

154   higher rates of glufosinate were required to cause substantial mortality for resistant (R and

155   R*) plants. The plants became dark grey from the middle of the leaves to the leaf tip, almost

156   burnt-like, with slight wilting, 24 h after treatment. The damaged area then extended in the

157   basipetal direction, developing necrosis over 14 days, turning wilted leaves from yellow into

158   brown. While the S plants die, the R and R* plants were observed to recover and grow again,

159   two weeks after treatment. The glufosinate LD_{50} for the R populations was 820 g ha^{-1} as

160   compared with 58 g ha^{-1} for the S population (Table 1), giving a LD_{50} R/S ratio of 14. This is

161   slightly higher than the previously reported LD_{50} R/S ratio of 7.6 (Jalaludin *et al*., 2010). The

162   difference may be due to different susceptible populations and experimental conditions used

163   in the two studies. The glufosinate GR_{50} for R population was found to be 156 g ha^{-1}, which

164   was about 5-fold greater than for the S population (Table 2). The selected R* population (the

165   progeny of plants surviving glufosinate rates of 1485 and 1980 g ha^{-1}) was only about 2-fold

166   more resistant to glufosinate relative to the original R population (Fig. 2A, Table 1, 2),

167   indicating the glufosinate-resistant sub population is still segregating.

168   *Tables 1 & 2 and Figs. 1 & 2 near here*

169

170   *Glyphosate resistance*

171   As expected, the S population was susceptible to glyphosate, with 100% mortality at the

172   glyphosate rate of 200 g ha^{-1} (Fig. 2B). However, the glufosinate resistant sub population R*

173   was found to be highly resistant to glyphosate, requiring an extremely high rate (25920 g ha^{-}

174   ^{1}) to cause substantial mortality (Fig. 2B). Based on the LD_{50} R/S ratio, the R* population

175   was more than 144-fold resistant to glyphosate (Table 1). While the R* plants survived high

176   glyphosate doses, their growth was affected. The GR_{50} for the R* and S population were 481

177   g ha^{-1} and 41 g ha^{-1}, respectively, resulting in the R* population being 12-fold more resistant

178   than the S population (Table 2). Therefore, in addition to glufosinate resistance, this R*

179   population had a high level of glyphosate resistance.

180   *Paraquat resistance*

181   The S population was, as expected, well controlled by paraquat at 375 g ha^{-1}, whereas control

182   of the R population required higher rates (Fig. 2C). Both S and R* plants displayed rapid

183   desiccation and necrosis following treatment. Similar to glufosinate-treated plants, the R*

184   plants recovered two weeks after treatment, while the S plants died. Based on the LD_{50} or

185   GR_{50} R/S ratio (Tables 1, 2), paraquat resistance in the purified glufosinate-resistant

186   population was confirmed, albeit at a low level (2 to 3-fold in relation to the used rate).

187   *Resistance to ACCase-inhibiting herbicides*

188   All ACCase herbicides examined (Table 3) caused 100% mortality in the S population at the

189   respective rates used. However, there was about 50% of the R* population surviving

190   haloxyfop-P-methyl, fluazifop-P-butyl or butroxydim. In contrast, the R* population

191   remained susceptible to sethoxydim, clethodim and imazapyr (Table 3).

192

193   *Table 3 near here*

194   *ACCase gene sequencing*

195   The plastidic ACCase gene sequences from a total of 9 individual plants surviving fluazifop-

196   P-butyl or butroxydim were analysed in comparison with those of the susceptible plants. The

197   primer pair ELEIN1781F/ELEIN1781R (Osuna *et al*., 2012) amplified a 600 bp DNA

198   fragment covering the known mutation site 1781 and the primer pair

199   ELEIN2027f/ELEIN2027r amplified an 832 bp fragment with the known mutation sites

200   1999, 2027, 2041, 2078, 2088 and 2096. Sequence alignment revealed an amino acid

201   substitution of Trp-2027-Cys in R individuals, resulting from a G to T change at the third

202   position of the Trp codon (TGG). The same mutation was also recently found in several other

203   fluazifop-resistant *E. indica* populations in Malaysia (Cha *et al.*, 2014). Generally, this

204   mutation has been known to confer resistance to ACCase-inhibiting

205   aryloxyphenoxypropionate herbicides (e.g. diclofop-methyl, fluazifop-P-butyl, haloxyfop-P-

206   methyl) (Délye, 2005; Powles & Yu, 2010). However it also confers resistance to ACCase-

207   inhibiting cyclohexanedione herbicides, for example, tralkoxydim in wild oats (Liu *et al*.,

208   2007). As the frequency of resistance to haloxyfop-P-methyl, fluazifop-P-butyl and

209   butroxydim is close to each other (around 50%, Table 3), it is very likely that the Trp-2027-

210   Cys mutation confers resistance to these three herbicides. Thus, this is the first case

211   associating the Trp-2027-Cys mutation with butroxydim resistance at the rate used.

212   **Discussion**

213   In this study, we confirmed the preliminary report on the evolution of resistance to

214   glufosinate in a Malaysian *E. indica* population (Jalaludin *et al*., 2010). The level of

215   glufosinate resistance determined for this population was modest (5- and 14-fold, based on

216   GR_{50} and LD_{50}, respectively), which is similar to the glufosinate-resistant *E. indica*

217   population reported by Chuah *et al*. (2010) (GR_{50} R/S ratio 3.4), and slightly higher than

218   glufosinate-resistant *Lolium perenne* populations in Oregon, USA (GR_{50} R/S ratios between

219   2.2 to 2.8) (Avila-Garcia & Mallory-Smith, 2011; Avila-Garcia *et al*., 2012). The level of

220   paraquat resistance in this population was also similar to that observed in a glufosinate- and

221   paraquat-resistant Malaysian *E.indica* population (Chuah *et al.*, 2010). It is worth noting that

222 usually GR_{50} R/S ratios are more variable than LD_{50} ratios, due to variations in growth

223 conditions and especially, the length of experiments. In this sense, LD_{50} R/S ratios would be

224 the better option to compare results across research groups.

225 Currently, documented glufosinate-resistance evolution is confined to a few *E. indica*

226 (Chuah *et al*., 2010; Jalaludin *et al*., 2010) and *L. perenne* populations (Avila-Garcia &

227 Mallory-Smith, 2011; Avila-Garcia *et al*., 2012) and all exhibit low to moderate levels of

228 glufosinate resistance. Few resistance mechanisms studies have been undertaken. In resistant

229 *L. perenne* populations, the resistance mechanism in one population was non-target-site based

230 (Avila-Garcia & Mallory-Smith, 2011), while in another population it was due to a target-site

231 mutation in the glutamine synthetase gene (Avila-Garcia *et al*., 2012). We have commenced

232 glufosinate resistance mechanism studies with this population.

233 Importantly, in addition to glufosinate resistance, individuals in this *E. indica*

234 population were also highly resistant to glyphosate (Fig. 2B; Table 1, 2). Resistant plants

235 survived very high glyphosate rates but suffered growth reduction, resulting in an R/S LD_{50}

236 ratio (144) much higher than the R/S GR_{50} ratio (12). The R/S ratios based on survival and

237 plant biomass were both higher than any previously reported evolved glyphosate resistance in

238 any weed species (Baerson *et al*., 2002; Mueller *et al*., 2011; Lee & Ngim, 2000; Gaines *et*

239 *al*., 2012; Culpepper *et al*., 2006). As is discussed above, we consider the LD_{50} value is more

240 accurate and meaningful in describing resistance levels, because it is less affected by

241 experimental conditionals (e.g. harvest time, growth competition) as compared with the GR_{50}

242 value. Nevertheless, the large difference in the R/S LD_{50} and GR_{50} ratio obtained for

243 glyphosate response in this *E. indica* population indicate that the potential glyphosate

244 resistance mechanism(s) may incur fitness cost in the presence of herbicide. This unusually

245 high level glyphosate resistance needs investigation. A few possible mechanism(s) are (1) a

246 new target-site EPSPS mutation, (2) multiple EPSPS mutations and (3) accumulation of

247   several known glyphosate resistance mechanisms (e.g. EPSPS gene mutation or

248   amplification, reduced glyphosate translocation or enhanced sequestration). We have initiated

249   studies to reveal the mechanistic basis of this very high level of glyphosate resistance.

250   Multiple resistance in *E. indica* has been reported previously. These multiple

251   resistance cases encompass at most, two different herbicide groups at any one time, e.g.

252   fluazifop-P-butyl and glyphosate (Heap, 2013) or glufosinate and paraquat (Chuah *et al*.,

253   2010). However, the current study is the first case where multiple resistance across four

254   dissimilar herbicide groups, glufosinate, glyphosate, paraquat and ACCase inhibitor

255   herbicides, is present in a single *E. indica* population. This is likely related to the herbicide

256   selection history of this population (involving application of at least paraquat, fluazifop-P-

257   butyl, glyphosate and up to 12 glufosinate applications per year). As resistance to glyphosate,

258   paraquat and ACCase-inhibiting herbicides was detected from a purified glufosinate-resistant

259   sub-population, it is very likely (although not examined) that multiple resistance is also

260   displayed at the individual level. Multiple resistance to glyphosate, paraquat and ACCase-

261   inhibiting herbicides in individual plants has been documented in *Lolium rigidum* L. due to

262   accumulation of multiple resistance mechanisms (Yu *et al*., 2007). This is the first global

263   report of a weed species with evolved resistance across all three of the world's non-selective

264   herbicides (glufosinate, glyphosate and paraquat). It is an unavoidable consequence of the

265   selection pressures resulting from over-reliance on herbicides for weed control, and therefore,

266   herbicides should be used wisely (e.g. in rotation or mixture) and in combination with other

267   non-chemical control options.

268   In summary, we have confirmed in an *E. indica* population the first case of multiple

269   resistance across the three non-selective herbicides, glufosinate, glyphosate and paraquat. The

270   same population also showed target-site resistance to ACCase-inhibiting herbicides, likely

271   due to the Trp-2027-Cys mutation. The evolution of multiple resistance to herbicides across

272 four different modes-of-action in this resistance-prone species is worrying, as it threatens the

273 world's most important herbicide (glyphosate) and its alternatives (glufosinate, paraquat) and

274 results in greatly reduced herbicide control options for the grower. Although other ACCase-

275 or ALS-inhibiting herbicides (e.g. sethoxydim, clethodim, imazapyr) still provide effective

276 short term control options, in the long run, additional diversity in weed control must be

277 added, to limit seed set of resistant *E. indica* plants.

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Table 1 Parameter estimates for logistic analysis of glufosinate, glyphosate and paraquat

dose-response survival data for the susceptible (S) and resistant (R) *Eleusine indica*

populations

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Population	a	b	$x_0 = \text{LD}_{50}$ (g a.i. ha^{-1})	R^2 (coefficient)	R/S ratio of LD$_{50}$
Glufosinate dose-response					
S	100.00 (0)	5.71 (0.23)	58 (0.81)	0.99	
R	100.00 (0)	2.42 (0.37)	820 (85.6)	0.93	14
[c]R*	100.00 (0)	2.3 (0.25)	1278 (63.9)	0.99	22
Glyphosate dose-response**					
S	100.00 (0)	15.28 (1.71)	148 (1.81)	0.98	
[c,d]R*	100.00 (0)	0.99 (0.1)	21274 (1773)	0.98	144
Paraquat dose-response					
S	100.00 (0)	3.76 (0.66)	98 (23.6)	0.97	
[c]R*	100.00 (0)	1.5 (0.2)	292 (27.9)	0.94	3

393 [c] R* refers to the selected glufosinate-resistant sub-population.

394 [d]*Glyphosate LD$_{50}$ is in g a.e. ha^{-1}.

395 Standard errors are in parentheses

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Table 2 Parameter estimates for logistic analysis of glufosinate, glyphosate and paraquat

dose-response biomass data for the susceptible (S) and resistant (R) *Eleusine indica*

populations

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Population	a	b	$x_0 = GR_{50}$ (g a.i. ha^{-1})	R^2 (coefficient)	R/S ratio of GR_{50}
Glufosinate dose-response					
S	100.00 (0)	2.23 (0.36)	31 (2.3)	0.94	
R	100.00 (0)	1.36 (0.17)	156 (17.4)	0.98	5
[c]R*	100.00 (0)	1.25 (0.25)	325 (37.1)	0.98	11
Glyphosate dose-response**					
S	100.00 (0)	1.7 (0.22)	41 (3.6)	0.92	
[c,d]R*	100.00 (0)	0.88 (0.09)	481 (55.6)	0.95	11.8
Paraquat dose-response					
S	100.00 (0)	3.22 (0.72)	52 (3.1)	0.95	
[c]R*	100.00 (0)	1.84 (0.29)	105 (8.4)	0.96	2

402 [c] R* refers to the selected glufosinate-resistant sub population.

403 [d]*GlyphosateGR$_{50}$ is in g a.e. ha^{-1}.

404 Standard errors are in parentheses

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Table 3 Percentage survival of the susceptible (S) and selected glufosinate-resistant (R*) sub populations of *E. indica* 21 days after treatment with various herbicides

Herbicide	Mean % survival	
	S	R*
ACCase inhibitor		
Fluazifop-P-butyl (210 g a.i. ha^{-1})	0	47
Haloxyfop-P-methyl (60 g a.i. ha^{-1})	0	51
Sethoxydim (230 g a.i. ha^{-1})	0	0
Clethodim (100 g a.i. ha^{-1})	0	0
Butroxydim (100 g a.i. ha^{-1})	0	49
ALS inhibitor		
Imazapyr (50 g a.i. ha^{-1})	0	0

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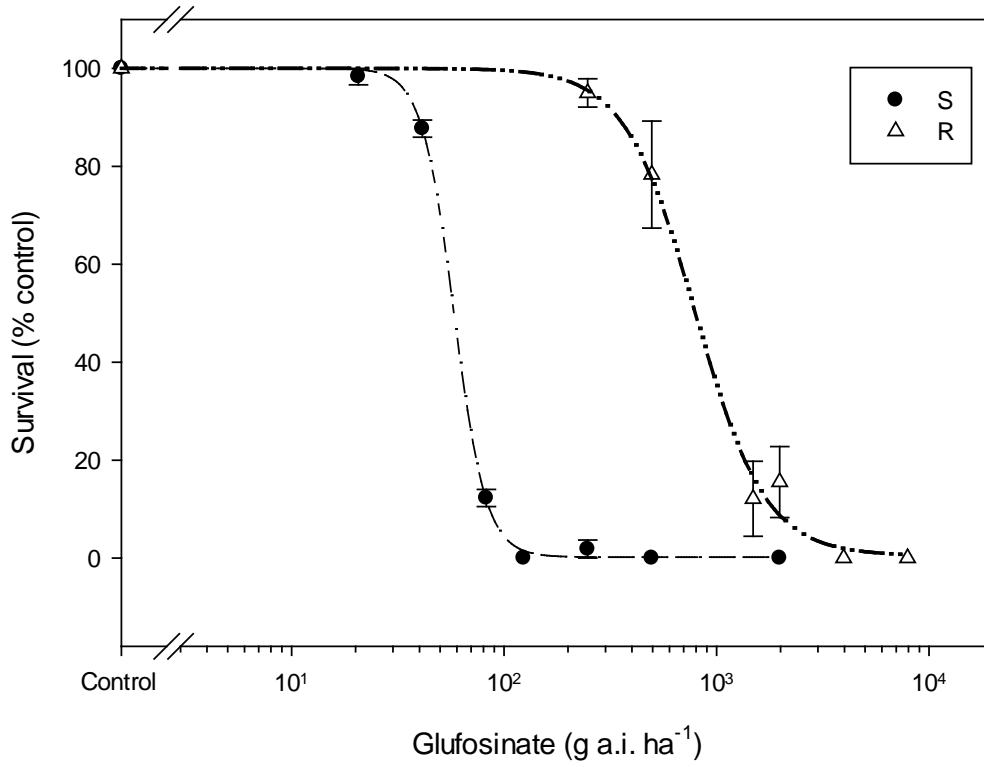
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Fig. 1 Glufosinate dose-response for survival of the susceptible (S) population and resistant

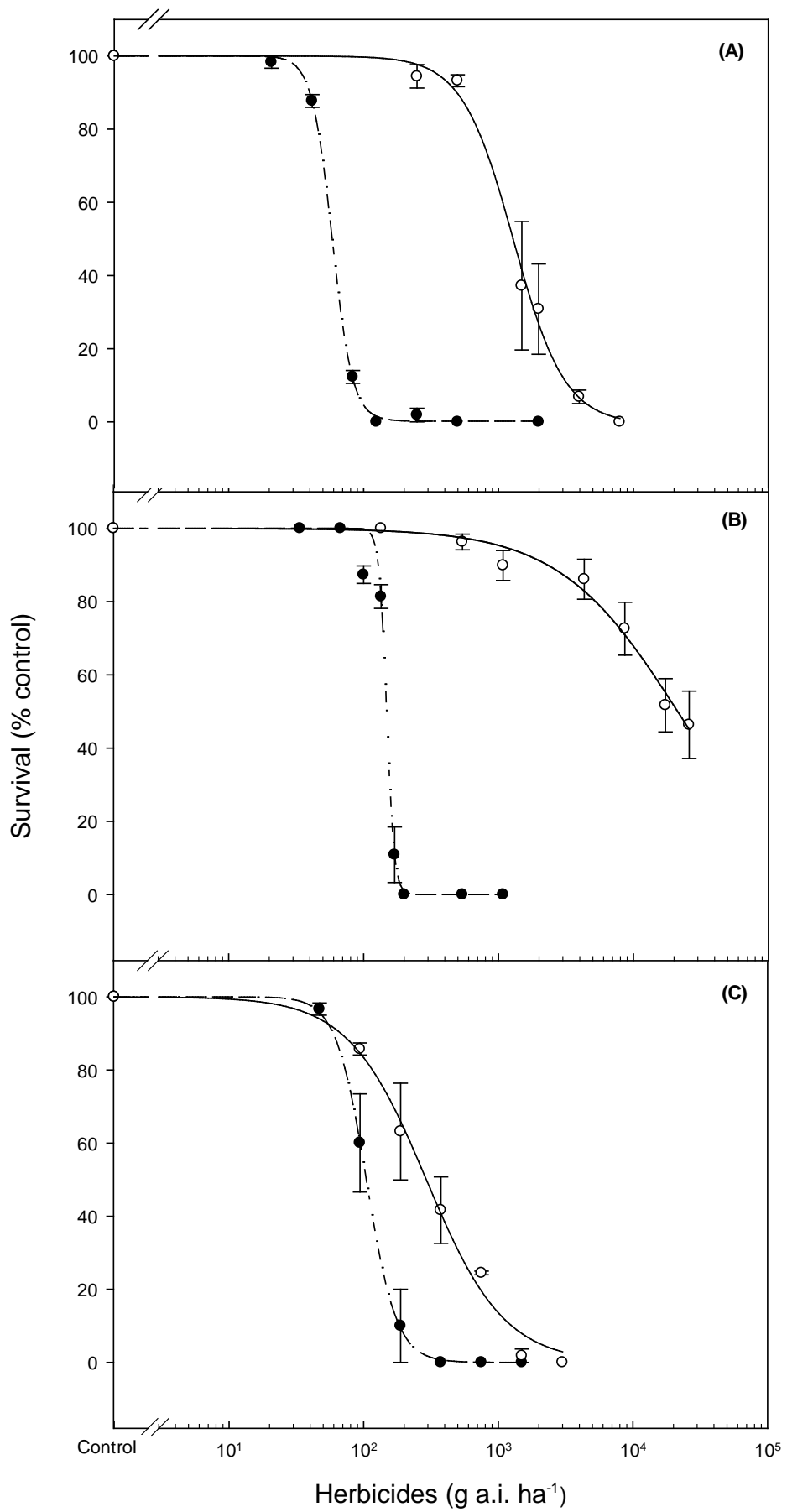(R) populations of *Eleusine indica*. Data were collected at 21 DAT.

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Survival (% control)

Herbicides (g a.i. ha⁻¹)

449   **Fig. 2** Survival response of the susceptible (closed circle; ●) and selected glufosinate-

450   resistant (opened circle; ○) R* sub populations of *Eleusine indica* to glufosinate (A),

451   glyphosate (B) and paraquat (C) treatment. Data were collected at 21 DAT. Glyphosate rates

452   are in g a.e. ha^{-1}.

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