Growth behavior and glyphosate resistance level in 10 biotypes of *Echinochloa colona* in Australia

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Abstract

Recently, poor control of *Echinochloa colona* with glyphosate has been reported in no-till agriculture systems of the northern grain region (NGR) of Australia. Two experiments were conducted using 10 biotypes of *E. colona* selected from the NGR of Australia to understand differences in their growth behavior and resistance pattern. Growth studies revealed that these biotypes differed in plant height (53-70 cm plant^{-1}), tiller production (30-52 tillers plant^{-1}), leaf production (124-186 leaves plant^{-1}) and seed head production (37-65 seed heads plant^{-1}). Days taken to seed heads and shoot biomass in these biotypes ranged between 40-48 d and 21-27 g plant^{-1}, respectively. Seed production in these biotypes ranged between 5380 and 10244 seeds plant^{-1}; lowest for biotype B17/25 and highest for biotype B17/13. Correlation studies revealed that seed number plant^{-1} had a positive correlation with plant height (r = 0.67), tiller number plant^{-1} (r = 0.89), leaf number plant^{-1} (r = 0.73), seed heads plant^{-1} (r = 0.78), seed head weight (r = 0.79), shoot biomass (r = 0.77) and root biomass (r = 0.46). The glyphosate dose-response study showed a wide range of responses in these biotypes and the glyphosate dose required to reduce 50% biomass (GR_{50} values) was estimated between 217 to 2159 g a.e. glyphosate ha^{-1}. GR_{50} values of biotypes B17/16, B 17/34 and B17/35 were 719, 2159 and 884 g ha^{-1}, respectively, making them 3, 10 and 4-fold resistant to glyphosate

compared with the susceptible biotype B17/37. Growth behavior and seed production potential in these biotypes had no correlation with the resistance index. These results suggest that some biotypes of *E. colona* are highly problematic; for example, biotype B17/34 was not only highly glyphosate-resistant, but also produced a high seed number (9300 seeds plant^{-1}). This study demonstrated that there is a possibility of great risk with the increased use of glyphosate for managing *E. colona* in the NGR of Australia. The results warrant integrated weed management strategies and improved stewardship guidelines are required for managing glyphosate-resistant biotypes of *E. colona* and to restrict further movement of resistant biotypes to other regions of Australia.

Keywords: Barnyard grass, Herbicide dose, Junglerice, Phenology, Seed number, Weed biomass

Introduction

Echinochloa colona (L.) Link (C_4 plant) has emerged as a major weed in summer crops in Australia and competes highly for water, sunlight and nutrients (1, 2). Worldwide, it is rated among the 10 most troublesome weeds. *E. colona* is widely distributed in the northern grain region (NGR) of Australia (3, 4, 5) and it costs Australian agriculture AU$ 14.7 million annually(6). Therefore, it affects the economy of Australian agriculture enormously.

Emergence of multiple cohorts in the summer season, along with high capacity for seed production and seed dispersal have allowed the spread of *E. colona* throughout the NGR of Australia. The seeds remain viable in the soil for more than one year, causing continuous recruitment (7). A significant portion of the fresh seeds of *E. colona* are dormant; therefore, retention of viability of original seed dispersal caused continuous reinfestation year after year.

In Australia, intraspecific variations in *E. colona* have been reported on the basis of genetic diversity (8). Such variations are referred to as clone, biotype or ecotype. Morphological studies of these biotypes may increase our knowledge further and identify how these biotypes adapt to climate change and play a role in invasiveness. A minor change in morphology or physiology of the plant may affect its adaptability in a changing climate and a large number of dispersed seeds in the field, combined with the ability of this weed to flower under a range of photoperiods, may contribute to its invasiveness (9).

In the NGR of Australia, *E. colona* is a very common weed in no-till fallow land and glyphosate spray is the most common management practice for managing this weed. Glyphosate was mostly used in orchards (high-value crops) when introduced in Australia during the 1970s, as it was relatively expensive (10). However, in the 1980s, its price declined, and its application became a common practice for weed control in a pre-seeding and fallow situation in Australia, which enabled the growers to adopt the conservation tillage practice. Glyphosate disrupts the shikmate pathways, reducing aromatic acid production via inhibition of the chloroplast enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Presently, control of some biotypes of *E. colona* in the NGR has become difficult with glyphosate as it has evolved resistance. The first case of glyphosate-resistant *E. colona* was reported in the NGR in 2007 (11). At present, 41 weed species have been reported as glyphosate-resistant worldwide (12). The evolved resistance may be due to intensive and repeated use of glyphosate (13, 14). *E. colona* has also evolved resistance to four other herbicide modes of action, in addition to glyphosate (15).

A better understanding of the differences between biotypes for control with glyphosate is essential for developing long-term strategies. Variation in growth, morphological and physiological characteristics may alter herbicide efficacy within a species.

Efficacy of glyphosate can be affected by plant species, biotype, plant development stage and environmental conditions (16).

Further, herbicide-resistant biotypes can spread from one area to another through pollen, seed or other propagules (17, 18). Therefore, it is important to understand characteristics of resistant biotypes of a specific area to make better decisions and long-term strategies for weed control (19, 20). Development of herbicide resistance in biotypes causes certain biochemical and physiological changes in the plant, which may be different from a susceptible biotype. A dose-response experiment is often conducted to assess the level of resistance in different biotypes. The dose-response experiment identifies a dose of a herbicide that provides a 50% reduction in shoot biomass (21).

In the NGR of Australia, there is variability in control of *E. colona* with glyphosate. We hypothesized that the dose required to reduce 50% growth of the plant (GR_{50} value) may vary between biotypes due to development of different levels of glyphosate resistance. It was also hypothesized that the reproduction potential of these biotypes may differ due to variability in the resistance factor. Information on resistant factor, and growth and reproduction behavior of these biotypes is limited in the NGR of Australia. Keeping these points in view, this study was planned to evaluate the growth, reproduction behavior and level of glyphosate resistance in different biotypes of *E. colona*. In this study, one experiment evaluated the growth and reproduction behavior of 10 biotypes of *E. colo*na from the NGR of Australia and another experiment evaluated the sensitivity of these biotypes to glyphosate.

Results and discussion

Growth and seed production

Amongst biotypes, the final plant height ranged from 53 to 70 cm, where the lowest was B17/35 and highest was B17/16. Biotypes B17/16, B17/17 and B17/25 attained a similar height, however, they were taller than biotypes B17/34 and B17/35 (Table 1). Tiller number among different biotypes ranged between 30 to 52 plant^{-1}, where the lowest was B17/25 and highest was B17/49 (Table 1). Biotypes B17/7, B17/12, B17/13 and B17/49 produced similar tiller numbers plant^{-1}, however, their tiller production was higher than biotypes B17/25 and B17/35. Leaf numbers in different biotypes varied from 124 to 192 leaves plant^{-1}, where the lowest was biotype B17/16 and highest was biotype B17/34. Leaf production (numbers plant^{-1}) remained similar for biotypes B17/34, B17/35 and B17/49, however, leaf production in these biotypes was higher than biotypes B17/16 and B17/25. All biotypes produced similar numbers of seed heads except for B17/25, which produced lower numbers than the other biotypes (Table 1).

The weight of seed heads among different biotypes varied from 6.2 to 9.9 g plant^{-1}. It was similar for biotypes B17/7, B17/12, B17/25, B17/34, B17/35, and B17/37 (6.2 to 7.9 g plant^{-1}), however, these biotypes had a lower seed head weight than biotypes B17/16 (9.8 g plant^{-1}) and B17/49 (9.9 g plant^{-1}). Shoot biomass among different biotypes ranged between 20.9 to 27.3 g plant^{-1} (Table 1). Shoot biomass remained similar for biotypes B17/13, B17/16 and B17/49, however, in these three biotypes, shoot biomass was significantly higher than biotypes B17/34, B17/35 and B17/37. Root biomass did not vary among biotypes (Table 1).

Time taken to seed head initiation in different biotypes varied from 40 to 48 d. Biotypes B17/7, B17/12, B17/13, and B17/17 took a similar time for seed head initiation (40-42 d) and produced seed heads earlier than biotypes B17/16, B17/25 and B17/35, of which B17/35 took the longest (48 d). Seed production in different biotypes varied from 5380 to 10244 seeds plant^{-1}; where the lowest was biotype B17/25 and highest was biotype B17/13.

123 Biotypes B17/12, B17/13, B17/34 and B17/49 produced a similar number of seeds (8298-
124 10244 plant^{-1}), with their seed production being higher than biotypes B17/25 and B17/35.
125 A linear positive correlation was found for seed number with plant height (r = 0.67),
126 tiller number plant^{-1} (r = 0.89), leaf number plant^{-1} (r = 0.73), seed heads plant^{-1} (r = 0.78),
127 seed head weight (r = 0.79), shoot biomass (r = 0.77) and root biomass (r = 0.46) (Table 2).
128 Shoot biomass had a negative relation with days taken to seed head initiation (r = -0.54)
129 (Table 2). Plant height, tiller production and seed head weight also had a negative relation
130 with days taken to seed head initiation. Root biomass had a positive correlation with tiller
131 production, leaf production and shoot biomass.
132 The results of this study demonstrated that characteristics like tall nature and high
133 tillering capacity allow *E. colona* biotypes to produce a high leaf number that resulted in a
134 large number of seed heads and seeds. Therefore, there is a need to target tiller production in
135 *E. colona* to reduce seed numbers. A recent study on crop-weed interference suggested that
136 crop competition could reduce tiller numbers in *E. colona* (22). In Australia, farmers are
137 following wide and skip row spacing in crops such as cotton (*Gossypium hirsutum* L.),
138 mungbean [*Vigna radiata* (L.) R. Wilczek] and sorghum [*Sorghum bicolor* (L.) Moench];
139 therefore, wide space between the rows could provide a better opportunity to *E. colona*
140 biotypes with a high tillering capacity nature as compared to when crops are sown in narrow
141 rows. In these environments (wide rows and fallows), *E. colona* could attain its high tillering
142 potential and could produce a high seed number. *E. colona* in the present study produced
143 tillers in the range of 39 to 52 plant^{-1}; however, in a previous study conducted in Greece, it
144 produced tillers in the range of 115 to 131 plant^{-1} (23). This difference could be due to
145 genotype x environment interactions and differential pot size. In the present study, we
146 observed that biotypes B17/13 and B17/49 had higher tillers than biotypes B17/25 and B
147 17/35. This also suggested that genotypes and environmental interactions played a role in

148 influencing tiller numbers per plant in *E. colona* biotypes. The regions where biotypes are of
149 high tillering capacity are expected to suffer a high crop yield loss due to high *E. colona*
150 competition.

151 The high seed number observed in biotypes B17/12, B17/13, B17/34 and B17/49 was
152 largely attributed to a high number of tillers, leaves, and seed heads. Our study also revealed
153 that seed head weight also influences seed number. The number of leaves and seed heads
154 were similar between biotypes B17/13 and B17/35; however, seed production was lower in
155 B17/35, which could be due to the lower tiller production and seed head weight in B17/35.
156 The time taken to seed head initiation in the present study was similar to a study conducted in
157 northern Greece, in which *E. colona* attained seed heads between 39 to 45 days after
158 transplanting (23). The biotype B17/35 (selected from the Moree region) took a longer time
159 for seed head initiation than other biotypes (Figure 1). In a previous study in South-East Asia,
160 12 *E. colona* biotypes were studied and it was found that time for seed heads in different
161 biotypes varied with latitude and plants from a high latitude attained seed heads earlier than
162 from a low latitude (24). This suggested that growth duration in different biotypes of *E.*
163 *colona* may vary with geographical location. In the present study, the negative relationship
164 between seed head initiation and seed number revealed that late-maturing biotypes produced
165 fewer seeds as was the case for biotypes B17/16 and B17/35 when compared with biotype
166 B17/13. Time taken to seed head initiation also had a negative relationship with plant height,
167 tiller number, and shoot biomass. These results suggest that diversity in *E. colona* traits could
168 result in differential responses to herbicides, cultural practices, and resistance evolution. For
169 example, the early vigor trait in *E. colona* is an important trait that could affect early crop-
170 weed competition (25) and therefore, management of such biotypes at an early stage is
171 required to increase crop production and reduce the weed seed bank in the soil.

In this study, *E. colona* biotypes differed in their seed production potential, which ranged between 5380 to 10240 seeds plant^{-1}. Differential seed production in *E. colona* biotypes could play an effective role in its spread and population establishment (26). High seed yields in biotypes B17/12, B17/13, B17/34 and B17/49 were largely based on a greater number of seed heads and leaf numbers plant^{-1}. High production of leaves in these biotypes probably maintained a better supply rate of carbon assimilates to seeds. In one study on *Brassica*, it was found that variation in the supply of carbon assimilates to seeds at or immediately after anthesis could cause a variation in seed production in different biotypes (27). Some authors also highlighted the role of the supply of carbon assimilates in determining the seed number in pea (*Pisum sativum* L.) plant (28).

The present study also revealed that tiller number per plant played a large role in seed production along with leaf number per plant. Biotype B17/35 had high leaf production but could not produce higher amounts of seeds like B17/13 and B17/34 did, because it had lower tiller production than B17/13 and B17/34. Although this study revealed that the supply of carbon assimilates after anthesis could be a major factor in determining seed production, we could not rule out the possibility of hormonal factors for variation in seed production in these biotypes. These results suggest that there is also a need to study nutritional and hormonal factors for variation in seed production in these biotypes (27). Our study (second experiment) also found that the GR_{50} value of glyphosate for these biotypes varied. These results suggest that in these biotypes, seed viability, seed persistence and fitness penalty may differ and therefore systematic studies need to be investigated. Such knowledge of seed production in these biotypes is required for understanding the evolution and spread of herbicide resistance particularly for herbicide-resistant biotypes.

197 **Response to glyphosate**

198 Out of 10 *E. colona* biotypes collected from the NGR of Australia, three biotypes (B17/16, B
199 17/34 and B17/35) had greater than 80% survival following treatments with 325 to 2600 g
200 a.e. ha^{-1} glyphosate. The probit analysis details for each biotype along with their level of
201 significance is presented in Table 3. The dose-response study of glyphosate for these biotypes
202 showed a wide range of responses (Figure 2). The GR_{50} value of the tested biotypes ranged
203 from 217 to 2159 g ha^{-1} (Figure 2). The susceptible biotype B17/37 was easily controlled
204 with glyphosate and had a GR_{50} of 217 g ha^{-1}, below the normal use rate of this herbicide
205 (650 g ha^{-1}). The GR_{50} values of biotypes B17/16, B17/34 and B17/35 were 719, 2159 and
206 884 g ha^{-1}, respectively, making them 3, 10 and 4-fold resistant to glyphosate compared with
207 the susceptible biotype B17/37. The most resistant biotype B17/34 was from the
208 Goondiwindi region, whereas the next most resistant biotypes, B17/35 and B17/16, were
209 from the Moree and Narrabri regions, respectively. This study has revealed that *E. colona*
210 biotypes in the NGR of Australia have different levels of resistance to glyphosate. No-till
211 farming is quite popular in the NGR of Australia for moisture conservation. Therefore,
212 growers use glyphosate in summer fallows to kill weeds and conserve moisture. Repeated and
213 intensive use of glyphosate in this region has evolved glyphosate resistance in these biotypes
214 (11). Risk of glyphosate resistance evolution for weeds is higher in those areas where
215 glyphosate has been used for a long time and with minimal soil disturbance (29). In Australia,
216 glyphosate-tolerant cotton varieties are very popular among cotton growers. Glyphosate-
217 resistant *E. colona* biotypes may create serious situations in that production environment. The
218 resistant factor for glyphosate in this study was similar to the first reported case of glyphosate
219 resistance in Australia (7 to 11-fold resistance compared with a susceptible population); but
220 that study was reported for rigid ryegrass (*Lolium rigidum* Gaud.) (30).

Earlier, glyphosate resistance in *E. colona* biotypes was also reported in Australia (11, 31). There are a number of mechanisms responsible for glyphosate resistance (32), and different mechanisms may result in a different level of resistance (33). Therefore, these studies suggests that these resistant biotypes may not carry the same resistance allele, which needs to be investigated. Many reports of glyphosate resistance in different weeds highlight that the reliance on glyphosate for weed control, in the long run, exerts a substantial selection pressure on weeds (34,35,36,37,38,39,40). Therefore, integrated weed control should be strengthened to reduce selection pressure on these resistant biotypes, particularly in cotton paddocks. It is quite possible that the mechanism of glyphosate resistance in Australian *E. colona* biotypes might be different from resistant *E. colona* biotypes reported from California (41) as Australian biotypes of *E. colona* have adapted to a dry environment. Therefore, a systematic study is required to understand the evolution of glyphosate resistance in these biotypes. The evolution of glyphosate resistance in tropical *E. colona* in Australia suggests that there is a large risk with increased use of glyphosate in fallows and improved stewardship guidelines for glyphosate use are required in the NGR of Australia.

The present study on *E. colona* biotypes has increased our understanding of the physiological basis of differences in seed production due to variations in morphological characteristics and resistance behavior. It highlighted that growth parameters such as high tiller production in *E. colona* biotypes leads to more leaves and in turn high seed production. The study further demonstrated that growth behavior and seed production potential in these biotypes had no correlation with the resistance index. However, this research has posed more questions than it has answered. This study suggested that biotypes such as B17/34 that are highly glyphosate-resistant, and also produced a high seed number (9300 seeds plant^{-1}) are very problematic. Therefore, systematic research on weed biology, physiology and resistance mechanism is required to answer these questions for better understanding. This study also

246 suggested that there is a need to understand the likelihood of resistance transfer from resistant
247 to susceptible biotypes through pollen-mediated gene flow and introgression. Such
248 knowledge could be useful in restricting the further spread of glyphosate-resistant biotypes of
249 *E. colona*.

250 **Materials and methods**

251 The study was conducted at the QAAFI weed science laboratory and screen house of the
252 University of Queensland, Gatton, Australia. Seeds of 10 different biotypes of *E. colona* were
253 collected from the NGR of Australia in March 2017. The coordinates of these biotypes are
254 depicted in Figure 1. The seeds of each biotype were cleaned and stored in shade.
255

256 **Growth response experiment**

257 In this experiment, 10 biotypes of *E. colona* were grown in pots replicated four times. The
258 pots were kept on benches placed outside the screen house. Pots were filled with potting mix
259 (Crasti & Company Pty Ltd, Sydney, Australia). Initially, 10 seeds were sown per pot at 1 cm
260 depth and after establishment, one plant per pot was maintained. The experiment was
261 conducted twice. The first run was started on 27 September 2018 and harvested on 6
262 December 2018. The second run was started on 3 December and harvested on 5 February
263 2019. Pots used in the experiment were 20 cm in height and arranged in a completely
264 randomized design. The pots were regularly irrigated.

265 At maturity, plant height was measured from the base of the plant to the tip of the
266 uppermost leaf of the plant. Days taken to seed head initiation was recorded in each pot. For
267 estimating seed production per head, two intact seed heads were chosen randomly from each
268 plant. For the total number of seeds, each rachilla segment (pedicel base) was counted and
269 then, averaged for seeds per head. At harvesting time, tiller numbers, leaf numbers and seed
270 heads per plant were also counted. Harvesting was done when ~80% seed heads matured.

271 At harvest, seed heads were separated from the plants for measuring shoot biomass.
272 After that, all aboveground shoot biomass from each plant was placed separately in a paper
273 bag and dried in an oven at 70 °C for 72 hours before being weighed. For root weight data,
274 pots containing potting mix with the root system were first dried in an oven at 70 °C for 72
275 hours. After that, roots were removed from each pot by shaking. Root biomass of each plant
276 was then measured. Drying of potting mix in an oven helped in the separation of the root
277 system from the potting mix.
278
279 **Glyphosate dose-response experiment**
280 Seeds of 10 biotypes were sown in pots (9 cm diameter and 10 cm height) filled with potting
281 mix (Crasti & Company Pty Ltd, Sydney, Australia). Initially, 10 seeds were sown per pot at
282 1 cm depth and after establishment, five plants per pot were maintained. Pots were kept in a
283 screen house under natural light and temperature conditions. The experimental design was a
284 factorial with four replicates where the first factor was biotype and the second factor was
285 glyphosate dose [0x (no herbicide; control), 0.5x, 1x, 2x, and 4x]. The 1x dose was the
286 recommended dose (650 g a.e. ha^{-1}) for glyphosate. The experiment was conducted twice.
287 The first run was started on 5 December 2018 and harvested on 14 January 2018. The second
288 run was started on 25 January 2019 and harvested on 6 March 2019. Glyphosate application
289 was done on 24 December 2018 in the first run and 13 February 2019 in the second run.
290 Plants were kept well-watered and fertilized.
291 Glyphosate was sprayed using a research track sprayer. Plants were treated at the 4-5
292 leaf stage using a spray volume of 108 L ha^{-1} and Teejet XR 110015 flat fan nozzles were
293 used. Plants were allowed to grow for 21 days after treatment (DAT) to determine glyphosate
294 sensitivity. Plant survival was assessed 21 DAT, and plant aboveground biomass was
295 harvested, dried for 72 hours at 70 °C, and weighed.

296

297 **Statistical analyses**

298 The first experiment was conducted in a completely randomized design and the second
299 experiment was conducted in a completely randomized design with a factorial arrangement.
300 In both experiments, there was no interaction between experimental runs and treatments;
301 therefore, the data of the two runs were pooled for ANOVA. All the data met assumptions of
302 normality of residuals and homogeneity of variance. Data of the first experiment were
303 subjected to analysis of variance (ANOVA) using the software Elementary Designs
304 Application 1.0 Beta (AgriStudy. com: www.agristudy.com) (verified with GENSTAT 16th
305 Edition; VSN International, Hemel Hempstead, UK). Treatment means were separated using
306 Fisher's protected LSD at $P \leq 0.05$.

307 For the second experiment, GR_{50} (the dose required to kill 50% of the growth of
308 plants) estimates were generated using Probit analysis [IBM SPSS Statistics 20.0 (SPSS,
309 Inc., Chicago, IL, USA)]. The level of significance was tested with a Chi-Square goodness
310 of fit test. When the calculated value of Chi-Square goodness of fit test was greater than the
311 table value, the null hypothesis was rejected and it was concluded that there was a significant
312 difference between the observed and the expected value and vice versa with values lower
313 than the table value. The resistance index (resistance/susceptibility ratio) was calculated on
314 the basis of the GR_{50} value to compare the resistance level among different biotypes.

315

316 **Author Contributions**

317 Conceptualization: Gulshan Mahajan, Bhagirath Singh Chauhan

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Data Availability Statement

All relevant data are within the paper and its Supporting Information files.

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Conflicts of Interest

The authors declare no conflicts of interest.

References

1 Holm L.G., Plucknett D.L., Pancho J.V., Herberger J.P., The World's Worst Weeds. Distribution and biology. Honolulu, University of Hawaii, 1977, 609 p.

2 Walker S.R., Taylor I.N., Milne G., Osten V.A., Hoque Z., Farquharson R.J., A survey of management and economic impact of weeds in dryland cotton cropping systems of subtropical Australia. Australian Journal of Experimental Agriculture, 2005. 45: p. 79-91.

3 Friend E. Queensland weed seeds. Department of Primary Industries, 1983. p. 206 .

4 Rew L.J., Medd R.W., Van de Ven. R., Gavin J.J., Robinson G.R., Tuitee M., Barnes J., Walker S. Weed species richness, density and relative abundance on farms in the subtropical grain region of Australia. Australian Journal of Experimental Agriculture, 2005. 45: p. 711-723.

5 Osten V.A., Walker S.R., Storrie A., Widderick M., Moylan P., Robinson G.R., Galea K. Survey of weed flora and management relative to cropping practices in the north-

346 eastern grain region of Australia. Australian Journal of Experimental Agriculture, 2007.
347 47: p. 57-70.
348 6 Llewellyn R., Ronning D., Clarke M., Mayfield A., Walker S., Ouzman J. Impact of weeds
349 in Australian grain production. *Grains Research and Development Corporation,*
350 Canberra, ACT, Australia, 2016. p. 112 .
351 7 Wu H., Walker S., Osten V., Taylor I., Sindel B. Emergence and persistence of barnyard
352 grass (*Echinochloa colona* (L.) Link) and its management options in sorghum. In *Weed*
353 *management: Balancing People, Planet, Profit. 14th Australian Weeds Conference,*
354 *Wagga Wagga, New South Wales, Australia, 6-9 September 2004: Papers and*
355 *Proceedings*
356 8 Nguyen T.H. Evolution and spread of glyphosate resistant barnyard grass *(Echinochloa*
357 *colona* (L.) Link) from Australia. 2015. (Doctoral dissertation). p. 151.
358 9 Swanton C.J., Huang J.Z., Shrestha A., Tollenaar M., Deen W., Rahimian H. Effects of
359 temperature and photoperiod on the phenological development of barnyard
360 grass. Agronomy Journal, 2000. 92: p. 1125-1134.
361 10 Duke S.O., Baerson S.R., Rimando A.M. Glyphosate. Encyclopedia of agrochemicals.
362 John Wiley & Sons, Inc. New York, USA, 2003.
363 11 Storrie A., Cook T., Boutsalis P., Penberthy D., Moylan P. Glyphosate resistance in
364 awnless barnyard grass (*Echinochloa colona* (L.) Link) and its implications for
365 Australian farming systems. 2008, 74 p. in R. D. van Klinken, et al. (2008) Proceedings
366 of the 16th Australian Weeds Conference. Queensland Weeds Society, Brisbane,
367 Queensland.
368 12 Heap I., Duke S.O. Overview of glyphosate-resistant weeds worldwide. Pest Management
369 Science, 2018, 74: p. 1040-1049.

370 13 Norsworthy J.K., Talbert R.E., Hoagland R.E. Chlorophyll fluorescence for rapid
371 detection of propanil-resistant barnyard grass (*Echinochloa crus-galli*). Weed Science,
372 1998. 46: p. 163-169.
373 14 Beckie H.J. Herbicide-resistant weed management: focus on glyphosate. Pest
374 Management Science, 2011. 67: p. 1037-1048.
375 15 Heap I. Global perspective of herbicide-resistant weeds. Pest Management Science, 2014.
376 70: p. 1306-1315.
377 16 Caseley J.C., Coupland D. Environmental and plant factors affecting glyphosate uptake,
378 movement and activity, in The Herbicide Glyphosate, ed. by Grossbard E and Atkinson
379 D. Butterworth & Co., London, UK, 1985, p. 92–123.
380 17 Christoffers M.J. Genetic aspects of herbicide-resistant weed management. Weed
381 Technology, 1999. 13: p. 647-652.
382 18 Délye C., Michel S., Bérard A., Chauvel B., Brunel D., Guillemin J.P., Dessaint F., Le
383 Corre V. Geographical variation in resistance to acetyl-coenzyme A
384 carboxylase-inhibiting herbicides across the range of the arable weed *Alopecurus*
385 *myosuroides* (black-grass). New Phytologist, 2010. 186: p. 1005-1017.
386 19 Thill D.C., Mallory-Smith C.A. The nature and consequence of weed spread in cropping
387 systems. Weed Science, 1997. 45:337-342.
388 20 Llewellyn R.S., Allen D.M. Expected mobility of herbicide resistance via weed seeds and
389 pollen in a Western Australian cropping region. Crop Protection, 2006. 25: p. 520-526.
390 21 Christoffoleti P.J. Curvas de dose-resposta de biótipos resistente e suscetível de *Bidens*
391 *pilosa* L. aos herbicidas inibidores da ALS. Scientia Agricola, 2002. 59: p: 513-519.
392 22 Mutti N.K., Mahajan G., Prashant J., Chauhan B.S. The response of glyphosate-resistant
393 and glyphosate-susceptible biotypes of junglerice (*Echinochloa colona*) to mungbean
394 interference. Weed Science, 2019. 67: p. 419-425.

395 23 Damalas C.A., Dhima K.V., Eleftherohorinos I.G. Morphological and physiological
396 variation among species of the genus *Echinochloa* in Northern Greece. Weed Science,
397 2008. 56: p. 416-423.
398 24 Chun J.C., Moody K. Differential competitiveness of *Echinochloa colona*
399 ecotypes. Korean Journal of Weed Science, 1987. 7: p. 247-256.
400 25 Smith A.E. Handbook of Weed Management Systems. 1st edn. New York: CRC Press,
401 1995. p. 758.
402 26 Thullen R.J., Keeley P.E. Seed production and germination in *Cyperus esculentus* and *C.*
403 *rotundus*. Weed Science, 1979. 27: p. 502–505.
404 27 Tayo T. The analysis of the physiological basis of yield in oil seed rape (*Brassica napus*
405 L.). 1974, Ph.D. thesis. University of Cambridge.
406 28 Meadley J.T., Milbourn G.M. The growth of vining peas: III. The effect of shading on
407 abscission of flowers and pods. Journal of Agricultural Science, 1977. 77: p.103-108.
408 29 Neve P., Diggle A.J., Smith F.P., Powles S.B. Simulating evolution of glyphosate
409 resistance in *Lolium rigidum* II: past, present and future glyphosate use in Australian
410 cropping. Weed Research, 2003. 43: p. 418-427.
411 30 Powles S.B., Lorraine-Colwill D.F., Dellow J.J., Preston C. Evolved resistance to
412 glyphosate in rigid ryegrass (*Lolium rigidum*) in Australia. Weed Science, 1998. 46: p.
413 604–607.
414 31 Gaines T.A., Ward S.M., Bukun B., Preston C., Leach J.E., Westra P. Interspecific
415 hybridization transfers a previously unknown glyphosate resistance mechanism in
416 *Amaranthus* species. Evolutionary Application, 2012. 5: p. 29–38.
417 32 Shaner D.L., Lindenmeyer R.B., Ostlie M.H. What have the mechanisms of resistance to
418 glyphosate taught us? Pest Management Science, 2012. 68: p. 3-9.

419 33 Preston C., Wakelin A.M., Dolman F.C., Bostamam Y., Boutsalis P. A decade of
420 glyphosate-resistant *Lolium* around the world: mechanisms, genes, fitness, and
421 agronomic management. Weed Science, 2009. 57: p. 435-441.
422 34 Binkholder K.M., Fresenburg B.S., Teuton T.C., Xiong X., Smeda R.J. Selection of
423 glyphosate-resistant annual bluegrass (*Poa annua*) on a golf course. Weed Science,
424 2011. 59: p. 286–289.
425 35 de Carvalho L.B., Cruz-Hipolito H., Gonzalez-Torralva F., da Costa Aguiar Alves P.L.,
426 Christoffoleti P.J., de Prado R. Detection of sourgrass (*Digitaria insularis*) biotypes
427 resistant to glyphosate in Brazil. Weed Science, 2011. 59: p. 171–176.
428 36 Dickson J.W., Scott R.C., Burgos N.R., Salas R.A., Smith K.L. Confirmation of
429 glyphosate-resistant italian ryegrass (*Lolium perenne* ssp. multiflorum) in Arkansas.
430 Weed Technology, 2011. 25: p. 674–679.
431 37 Light G.G., Mohammed M.Y., Dotray P.A., Chandler J.M., Wright R.J. Glyphosate-
432 resistant common waterhemp (*Amaranthus rudis*) confirmed in Texas. Weed
433 Technology, 2011. 25: p. 480–485.
434 38 Mueller T.C., Barnett K.A., Brosnan J.T., Steckel L.E. Glyphosate resistant goosegrass
435 (*Eleusine indica*) confirmed in Tennessee. Weed Science 2011. 59: p. 562–566.
436 39 Norsworthy J.K., Riar D., Jha P., Scott R.C. Confirmation, control, and physiology of
437 glyphosate-resistant giant ragweed (*Ambrosia trifida*) in Arkansas. Weed Technology,
438 2011. 25: p. 430–435.
439 40 Riar D.S., Norsworthy J.K., Johnson D.B., Scott R.C., Bagavathiannan M. Glyphosate
440 resistance in a johnsongrass (*Sorghum halepense*) biotype from Arkansas. Weed
441 Science, 2011. 59: p. 299–304.
442 41 Alarcón-Reverte R., García A., Watson S.B., Abdallah I., Sabaté S., Hernández M.J.,
443 Dayan F.E., Fischer A.J. Concerted action of target-site mutations and high EPSPS

444 activity in glyphosate-resistant junglerice (*Echinochloa colona*) from California. Pest
445 Management Science, 2015. 71: p. 996-1007.

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Table 1. Morphological traits and seed production potential of different biotypes of *Echinochloa colona*

Biotype	Plant height (cm)	Tiller (number plant^{-1})	Leaf (number plant^{-1})	Seed head (number plant^{-1})	Seed head weight (g plant^{-1})	Shoot biomass (g plant^{-1})	Root biomass (g plant^{-1})	Days to seed head initiation (d)	Seed production (number plant^{-1})
B17/7	62.9	47	134.7	52.7	7.7	24.8	12.7	42	7022
B17/12	61.2	47	163.6	63.9	7.5	24.2	13.0	41	8837
B17/13	63.7	50	156.3	59.4	8.5	25.1	18.0	40	10244
B17/16	70.3	40	124.1	53.5	9.8	27.3	10.1	45	6986
B17/17	66.9	41	134.5	60.5	8.1	22.7	9.7	42	7801
B17/25	66.7	30	132.1	36.6	6.2	22.1	13.7	45	5380
B17/34	56.2	43	192.1	62.9	7.9	20.9	12.5	44	9295
B17/35	53.1	39	185.9	65.1	6.2	20.9	16.1	48	6130
B17/37	63.2	43	162.1	59.0	7.5	21.1	12.4	43	6387
B17/49	62.9	52	165.7	63.7	9.9	25.4	11.9	44	8298
LSD (0.05)	5.7	7.0	30.5	14.2	1.7	3.8	NS	2.7	2136

NS: nonsignificant

Table 2. Correlation of morphological traits with seed number in different biotypes of *Echinochloa colona*

Parameter	Plant height (cm)	Tiller (number plant^{-1})	Leaf (number plant^{-1})	Seed head (number plant^{-1})	Seed head weight (g)	Shoot biomass (g plant^{-1})	Root biomass (g plant^{-1})	Days to seed head initiation (d)	Seed production (number plant^{-1})
Tiller (number plant^{-1})	1								
Leaf (number plant^{-1})	0.77*								
Seed head (number plant^{-1})	0.55*	0.77*							
Seed head weight (g plant^{-1})	0.59*	0.87*	0.87*						
Seed head weight (g plant^{-1})	0.82*	0.84*	0.51*	0.68*					
Shoot biomass (g plant^{-1})	0.96*	0.88*	0.70*	0.72*	0.83*				
Root biomass (g plant^{-1})	0.36	0.47*	0.52*	0.39	0.16	0.47*			
Days to seed head initiation (d)	-0.54*	-0.65*	-0.26	-0.42	-0.55*	-0.54*	-0.16		
Seed production (number plant^{-1})	0.66*	0.88*	0.70*	0.78*	0.73*	0.78*	0.53*	-0.68*	1

Critical value of *r* at 5% = 0.44; * indicates significant relation

Table 3. Probit analysis detail [Intercept a + b*x* (covariate *x* are transformed using the base 10.0 logarithm)] for different *Echinochloa colona* biotypes

Biotype	***a* Intercept**	***b* Estimate**	**Pearson Goodness- of-fit *Chi square***	**Significance level (*Chi square*)**
B17/7	-9.23	3.95	0.95	0.62
B17/12	-9.37	3.94	3.98	0.14
B17/13	-9.25	3.63	2.42	0.30
B17/16	-1.55	0.54	0.78	0.68
B17/17	-7.76	3.31	1.10	0.58
B17/25	-13.8	5.51	0.01	0.99
B17/34	-5.25	1.57	5.68	0.06
B17/35	-2.94	0.99	5.48	0.06
B17/37	-3.11	1.33	3.19	0.20
B17/49	-4.41	1.66	3.25	0.20

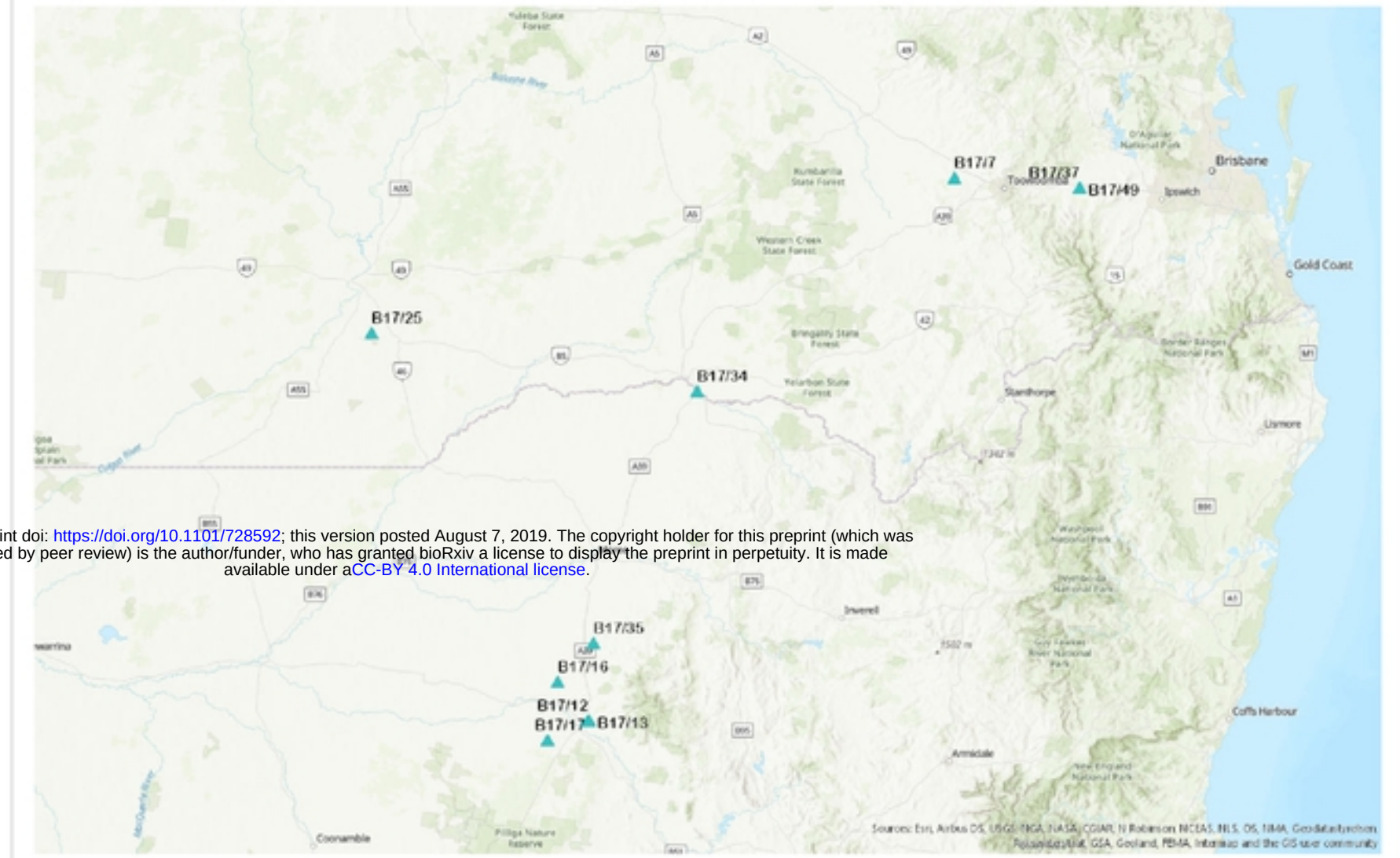

Figure 1. Location of 10 biotypes of *Echinochloa colona* selected from the northern grain region of Australia

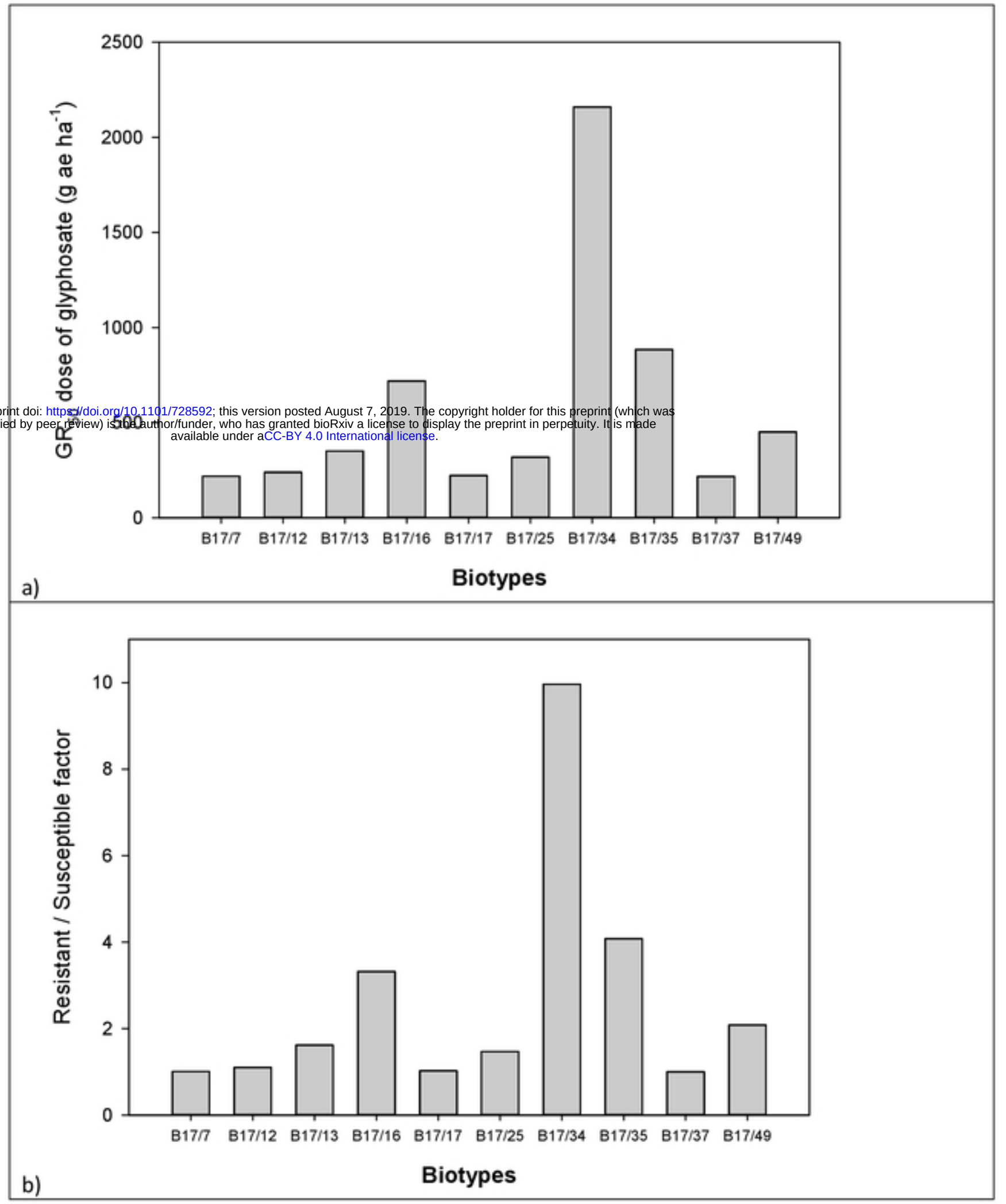

Figure 2: a) GR_{50} dose of glyphosate and b) glyphosate-resistant/susceptible factor in different biotypes of *Echinochloa colona*.