Physiological Fitness Cost Associated with Glyphosate Resistance in *Echinochloa colona*: Seed Germination Ecology

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ABSTRACT

The expression of fitness cost associated with herbicide resistance in weeds is not universal and may vary during different life stages. Hence, an analysis of the fitness cost of a glyphosate-resistant *Echinochloa colona* at the seed stage was conducted. Seed germination ecology of glyphosate-susceptible and -resistant *E. colona* was studied to examine the physiological fitness cost between the S (susceptible) and R (resistant) phenotypes. The plant material was selected from within one segregating glyphosate-resistant *E. colona* population to minimise allelic interference from other fitness-related loci. Experiments were conducted in a growth incubator at alternating temperatures of 30/20°C day/night with a 12-h photoperiod. Seed dormancy and germination were also tested under constant darkness at the same temperature regime. Seeds of both the S and R phenotypes kept at warmer temperatures (after-ripening dry storage at 15 to 35°C) or on the ground surface outdoors for 14 days, germinated better (with more than 95% of seeds had germinated) than those seeds (about 20% germination) that were kept at lower temperature (8°C) for the same period of storage. Light and longer dry after-ripening times increased the rate of seed dormancy release and germination (almost 100% seed germination) for both the S and R phenotypes. Equal decline in seedling emergence in both the S and R phenotypes was evident with increasing soil burial depth ($P > 0.05$). Thus, the S and R phenotypes exhibited similar characteristics of seed dormancy release, germination and seedling emergence. As there is lack of physiological fitness cost in the R phenotype of *E. colona* at the seed and early seedling stage, it remains a challenge to develop specific control measures for the R phenotype. An alternative herbicide with different mode of action should be integrated with other weed management approach such as cultural, mechanical and biological control to reduce sole reliance of glyphosate in controlling the R phenotype of this *E. colona* population.

Keywords: *Echinochloa colona*, fitness cost, glyphosate, seed germination, seedling emergence

INTRODUCTION

Plants possessing traits for resistance to herbicides may display an ecological disadvantage compared to susceptible plants in a herbicide-free environment (Vila-Aiub et al., 2009). Within the context of herbicide resistance evolution, plants carrying genetic traits conferring herbicide resistance can express a fitness cost in the absence of herbicide selection. However, the expression of fitness costs is not universal and has been shown to depend on the considered resistance gene and specific allele, the genetic dominance of such a resistance cost, the genetic background and the ecological environment (Roux et al., 2004; Vila-Aiub et al., 2009, 2011, 2019; Yu et al., 2010).

Physiological fitness costs are generally mediated by pleiotropic effects on plant physiology which can affect resource partition, growth rate, photosynthesis, phenology, seed germination and dormancy, and tolerance to abiotic factors (Vila-Aiub et al., 2009). Some environmental conditions may

trigger the maximum expression of a cost while others may mask and make them negligible (Ashigh and Tardif, 2011). Once a physiological cost is detected in a set of environmental conditions, it may be possible to re-create and exploit that cost in an ecological environment (e.g. under crop competition) resulting in a control benefit from a resistance management view.

Resistance to glyphosate is increasingly evolving in many weed species and this poses a severe risk to the most widely used herbicide in agriculture (Duke and Powles, 2008). One of the weed species that has evolved resistance to glyphosate was *Echinochloa colona* (L.) Link (Gaines et al., 2012). *E. colona* is commonly known as awnless barnyardgrass or junglerice. This species has been ranked among the top four most important weeds in the world due to its widespread infestation across six different continents (Holm et al., 1991). *E. colona* is a C4 (summer) annual species that grows aggressively in well-drained soil and under full sunlight (Holm et al., 1991). It is widely distributed in the warm regions of Asia, Africa, Australia and the northern part of South America. It occurs commonly in rice fields and in plantation crops, orchards and vegetable farms, and in non-cropping areas such as along fence lines, railway rights of way and roadsides (Holm et al., 1991). It can be propagated through seed and vegetative parts, where rooting is often found at the lower nodes.

Despite the importance of glyphosate resistance, no studies have been explored on its pleiotropic effects on seed germination traits. This study reported the assessment of physiological fitness costs associated with glyphosate resistance in *E. colona* from Western Australia (WA). Particularly, seed germination and seedling emergence responses at various thermal and light conditions were evaluated in glyphosate-susceptible and -resistant plants.

MATERIALS AND METHODS

Seed source and preparation

To minimise variation at fitness-related loci which are not directly linked to the mechanism(s) endowing herbicide resistance, efforts were made in the selection of plant materials for this study, where the S and R phenotypes were selected from within a segregating glyphosate-resistant *E. colona* population. Both the susceptible (S) and resistant (R) phenotypes of *E. colona* grown from seeds originally collected in the field from the Tropical Ord River region of Western Australia in 2009 and identified according to the procedure described in Goh et al. (2016) were subjected to glyphosate selection in order to further purify the desired plant material. A total of 15 identified S and R plants were then selected from the untreated corresponding cloned parental plants and these were individually transplanted into large plastic pots (24.5 cm diameter and 27.5 cm height). The seeds produced by these 15 plants grown in the same environment through self-pollination, were used in the subsequent studies. All stages of plant growth and seed production were conducted outdoors at the University of Western Australia (S 31°59’; E 115°49’) with an average temperature of 27°C and a 13 to 14 h photoperiod.

Seed after-ripening and germination conditions

The seed dormancy release study was repeated in two consecutive growing seasons, where fresh seeds were produced and collected each year (hereinafter denoted as Season 1 and Season 2). Seeds for Season 1 were harvested in autumn (April 2013; 5-month old plants) while those for Season 2 were harvested during summer (February 2014; 3-month old plants). Freshly matured seeds harvested from each S and R phenotype were immediately divided into five equal samples and maintained at constant temperatures at 8, 15, 25 or 35°C as well as under natural fluctuating temperature conditions (placed in sunlight on an outdoors soil surface undisturbed by other cultivation practices), respectively. High germination of seeds, imbibed under optimal conditions, had essentially reached 100% after two weeks (Figure 1). Seeds were kept in small permeable mesh sachets which allowed sufficient oxygen exchange. The average diurnal temperature range outdoors was 23 to 27°C with a 13- and 14-h photoperiod, respectively.

Effect of seed storage temperature (dry after-ripening) on seed dormancy release

Four replicates of 50 dry-stored seeds for each phenotype exposed to a different after-ripening temperature regime, were incubated in a growth incubator set at alternating temperatures of 30/20°C day/night with either a 12-h photoperiod (hereinafter denoted as 12-h light) or in constant darkness on 1.0% (w/v) agar-solidified water in 9 cm diameter petri dishes. These conditions were selected because preliminary results showed associated optimal germination rates in non-dormant seeds for both S- and R-*E. colona* (data not shown). The germination light intensity was 101 μmol m^{-2} s^{-1}, produced by cool white fluorescent tubes as measured with a quantum meter (Model QMSW, Apogee Instruments, USA). Petri dishes kept in the dark were wrapped with aluminium foil to exclude light. All petri dishes were kept in clear sealing bags to reduce water evaporation. Germination was evaluated daily for the first 7 days and at weekly intervals for the subsequent readings, for a period of 42 days after seed imbibition. Seeds were considered to have germinated when radicles with more than 1 mm in length had emerged. Non-germinated seeds were subjected to a tetrazolium test (ISTA, 2005) to determine their viability at the end of the incubation period. Non-germinated seeds were longitudinally sliced to expose the endosperm and incubated in 1% (w/v) tetrazolium chloride solution for 24 h in the dark at 30°C. Seeds were considered viable if pink staining of the embryo and aleurone was observed.

Seed germination of glyphosate-susceptible and -resistant *E. colona*

Non-dormant seeds exposed to after-ripening for a month at 35°C were used in this seed germination study. Viability of seeds for both S and R phenotypes was tested using tetrazolium chloride prior to the germination study. Four replicates of 50 seeds for each phenotype were placed on 1.0% (w/v) agar-solidified water in 9 cm diameter petri dishes and incubated in the growth chamber at the alternating temperature 30/20°C day/night with 12-h light or constant darkness, and germination was assessed as described above.

Effect of burial depth on seedling emergence of glyphosate-susceptible and -resistant *E. colona*

Four replicates of 20 non-dormant seeds (total n = 80) collected from each of the 15 individual plants of S and R, which displayed similar mean seed mass and no significant differences in seed germination percentage (100%), were placed at different burial depths of 0.5, 2, 4, 6, 8 or 10 cm and covered with standard potting mixture in 12 x 12 x 21 cm plastic square tube pots. The same quantity of soil to fill each pot ensured uniform texture to avoid differential resistance to seedling emergence. Pots were placed in the plant growth chamber at the alternating temperature of 30/20°C day/night with a mean light intensity of 104 μmol m^{-2} s^{-1} and air humidity of 47% and were kept well-watered. Emerged seedlings were counted daily until no further emergence was recorded. Seeds were considered emerged when the coleoptile was visible at the soil surface. After emergence tests, soil was removed from pots to determine the fate of non-germinated seeds (dormant or germinated without emergence).

Statistical analysis

For each study, there were four replicates per treatment per phenotype. All seed germination studies were arranged in a completely randomized design and were conducted twice. The cumulative number of germinated seeds for the seed dormancy release and seed germination studies, as well as cumulative emergence for the seedling emergence study, were recorded as a percentage of the total number of viable seeds used in each replication. To satisfy the assumptions of independent and normally distributed variance, a logarithmic transformation (log (x+1)) of cumulative germination, and seedling percentages was necessary. All log-transformed data and mean emergence time were subjected to analysis of variance

(ANOVA). Means separation was determined by the Tukey test using the Proc ANOVA procedure of the SAS package (SAS 9.3, SAS Institute Inc., Cary, NC, USA).

For the seed dormancy release study, a three- or four-way analysis of variance (ANOVA) (the main effects or variables were Experiment, Light, Phenotype and After-ripening Temperature as well as their interactions) was conducted. Seed germination at after-ripening time (t) = 0 (immediately after harvest), was significantly different between experiments and at different light intensities (Exp x Light) as well as the interaction between light and phenotype (Light x Phenotype). Two weeks after-ripening similarly there was significant interaction between experiment and temperature (Exp x After-ripening Temperature), experiment and light intensity (Exp x Light), temperature and light (After-ripening Temperature x Light) and all the three combinations (Exp x After-ripening Temperature x Light). Thus, the total percentage of cumulative germination was plotted separately for each experiment.

Data from the seed germination and seedling emergence studies were subjected to ANOVA (the main effects or variable were Exp, Light, Phenotype and their interaction effects for the former study, and Exp, Burial Depth, Phenotype and their interaction effects for the latter study). The two experiments did not differ in all variables for seed germination and seedling emergence and no interaction effects were found. Thus, the data were pooled. The analysis results obtained from the transformed data were similar to those for the original untransformed analyses. Therefore, all results reported here are from the original untransformed data.

For the seed dormancy release study, all cumulative germination patterns under light treatment at each after-ripening temperature were fitted to a three-parameter Gompertz function, viz. $y = ae^{-e^{\frac{-(t-t_{50})}{b}}}$ using SigmaPlot 12.0 software (Systat Software, Inc, CA, USA), whereas cumulative germination data for the darkness treatment were fitted to either the three-parameter Hill function, viz. $y = \frac{a\,(t^b)}{(t_{50})^b + t^b}$ or exponential function, viz. $y = ae^{\frac{b}{t}}$, where for the corresponding regression models, y is the cumulative germination (%) at days after seed imbibition (t), a is the asymptote or maximum cumulative germination, b is the slope of the curve and t_{50} is the time (in days) required to achieve 50% of the maximum cumulative germination (or the time in days required for dormancy release in 50% of the imbibed seeds at each treatment).

A functional three-parameter sigmoidal model was fitted to the cumulative germination percentage for the seed germination study and the model fitted was: $y = \frac{a}{[1+e^{\frac{-(t-t_{50})}{b}}]}$, where y is the cumulative germination (%) at days after seed imbibition (t), a is the maximum cumulative germination, b is the slope of the curve and t_{50} is the time (in days) to reach 50% of the maximum cumulative germination.

Cumulative seedling emergence (in percentage) of both phenotypes over the studied period was fitted to three-parameter Hill function for each burial depth treatment. The fitted model was: $y = \frac{a\,(t^b)}{(t_{50})^b + t^b}$, where y is the cumulative seedling emergence (%) at time t (days after seed sowing), a is the asymptote or maximum cumulative seedling emergence, b is the slope of the curve and t_{50} is the time (in days) required for 50% of viable seeds to emerge at each burial depth.

RESULTS AND DISCUSSION

Seed dormancy release study

This study revealed that susceptible (S) and resistant (R) *E. colona* seeds isolated from within a glyphosate resistant population of *E. colona* have very similar characteristics of seed dormancy release,

germination and seedling emergence. It was concluded that at the seed level, no physiological fitness cost was evident in this glyphosate R phenotype of *E. colona*. An understanding of the dormancy, germination and emergence of seeds of glyphosate-resistant *E. colona* could help predict its fitness and potential ecological succession into new areas. This information is valuable in determining the expression of fitness costs at this particular life phase and developing effective measures to manage this glyphosate-resistant weed.

Seed dormancy release was determined using the percentage of seed germination after 14 days of after-ripening at various temperature regimes. Generally, there was no significant difference in seed dormancy release between the S and R phenotypes across the different after-ripening temperatures regardless of light and darkness (Figure 1). Higher rates of seed dormancy release and germination of both S and R phenotypes were observed under light condition and longer dry after-ripening times. Cumulative germination after 14 days of after-ripening temperatures of 15, 25 or 35°C, or exposed to outdoors conditions under a fluctuating 12-h photoperiod, was consistently very high in both experiments. Seed dormancy release was, however, relatively slower at a low after-ripening temperature of 8°C (Figure 1). Seeds of S and R phenotypes kept at warmer temperatures (after-ripening dry storage at 15-35°C) or on the ground surface outdoors for 14 days, germinated better than those seeds that were kept at lower temperature (8°C) for the same period of storage ($P < 0.05$).

Following 14 days seed after-ripening, both phenotypes showed poor germination under constant darkness (less than 40%) across most of the after-ripening temperatures, with the exception of the 35°C after-ripening treatment in Season 1 (Figure 1). However, the proportion of seeds which germinated in darkness increased with an increasing after-ripening time. Lower germination was observed in the 35°C-after-ripened S phenotype compared to the R phenotype in darkness in Season 1 (Figure 1a) and this may be attributed to the presence of a greater number of dormant seeds in the population at harvest in the S phenotype (data not shown). The process of dormancy-release in seeds of *E. colona*, in both the glyphosate-susceptible (S) and -resistant (R) phenotypes, was more rapid at higher after-ripening temperatures, although seeds that had been after-ripened for 14 days retained a requirement for light to achieve optimal germination. This result was consistent with previous studies conducted by Chauhan and Johnson (2009), and Kovach et al. (2010) where germination of *E. colona* was found to be stimulated by light.

The low level of seed germination when exposed to a low after-ripening temperature (8°C) suggests that as expected for a tropical species, *E. colona* would remain dormant in environments with cool temperatures. In the present study, non-after ripened *E. colona* seeds harvested in autumn (Season 1) displayed a higher and faster germination level than that of the seeds harvested during summer (Season 2). This was likely due to the fact that seeds maturing during the long summer experience a longer period of after-ripening whilst still on the mother plant, where the seeds exhibit lower seed dormancy at harvest in Season 1 compared to those seeds harvested in Season 2. Similar findings were observed in a study conducted with *Lolium rigidum* Gaudin seeds. Fresh matured seeds of different populations were collected from wheat crops in two different locations at Merredin and Wongan Hills (approximately 160 km apart) in Western Australia during summer (Steadman et al., 2003). Seeds collected in Merredin had generally lower seed dormancy at harvest and displayed 45% germination compared with seeds from Wongan Hills with 20% germination (Steadman et al., 2003). In line with these results, Holm et al. (1991) reported that *E. colona* had a short period of seed dormancy, with the dormancy vanishing in less than 8 weeks of dry storage after harvesting. The light requirement for germination of both the S and R *E. colona* seeds was reduced after 28 days of dry storage at 35°C for both Season 1 and Season 2, in which final germination had increased 2- to 2.5-fold from an average of about 39-48% after 14 days of dry storage to an approximately 96-100% after dry storage for 28 days in darkness (data not shown).

Seed germination study

Under a 12-h light regime, there was no significant difference (P = 0.7) in seed germination between the S and R phenotypes. Meanwhile the S phenotype had a significant lower maximum seed germination percentage in darkness than for the R phenotype (Figure 2). When dark-treated seeds were subsequently exposed to 12-h light, germination of both phenotypes reached 100% (data not shown). This indicated that seed germination was similar for both the S and R phenotypes at the end of the study. It has been shown that the germination percentage of *E. colona* seeds incubated in the dark increased after the seeds had been in dry storage for 2 months and reached a similar level of germination to that of freshly-harvested seeds imbibed under white light (van Rooden et al., 1970).

Seedling emergence study

As expected, final germination percentage differed significantly across burial depths from 0.5 to 10 cm, but overall effects were similar for seeds of both the S and R phenotypes (P > 0.05). Based on the fitted model, similar seedling emergence rate and time required to reach 50% emergence were found between the S and R phenotypes at each burial depth over a period of ten days (Figure 3). Other species such as *Artemisia tridentata* Nutt. and *Eragrostis ferruginea* (Thunb.) P.Beauv. have exhibited a gradual reduction in photo-requirement for seed germination in darkness with increasing after-ripening time (Meyer et al., 1990). The results from the present seedling emergence study, which revealed that more than 90% of the non-dormant seeds of both the S and R phenotypes had germinated without light even at the burial depth of 10 cm, further supported these findings.

Comparison of seed dormancy and germination in the S and R phenotypes

It has been emphasised that plant fitness studies associated with evolved herbicide resistance should be conducted using resistant and susceptible genotypes or phenotypes sharing a similar genetic background except for the resistance gene/s (Darmency et al., 2015; Vila-Aiub et al., 2009, 2015). To minimise variation at fitness-related loci which are not directly linked to the mechanism(s) endowing herbicide resistance, efforts were made in the selection of plant materials for this study, where the S and R phenotypes were selected from within a segregating glyphosate-resistant *E. colona* population (Goh et al., 2016). Since the R phenotype of this single population was unlikely to be due to target-site glyphosate resistance mechanisms (Goh et al., 2018), in which case a confounding effect of heterozygous alleles could have taken place, it was reasonable to conclude that the seed germination ecology characterised in this study would reflect the true ecological measure of resistance.

To date, data on the seed germination ecology associated with glyphosate-resistance is scarce. In this present study, no difference was found in seed dormancy release and germination patterns between the S and R phenotypes. Thus far, comparisons of the seed germination ecology of glyphosate-susceptible and -resistant weed populations have been conducted with *Eleusine indica* (L.) Gaertn., *Ambrosia artemisiifolia* L. and *Ambrosia trifida* L. (Dinelli et al., 2013; Ismail et al., 2002). Results from these studies exhibited different responses in germination, seedling emergence and/or seed dormancy. However, these studies did not control the genetic background of the plant materials as two different genetically unrelated populations were used. Moreover, it was reported that the seeds were scarified or cold stratified prior to the seed germination study. It has been well established that any change or modification on the seed itself with pre-treatments (i.e. through physical, mechanical or chemical etc. approaches) can affect the rate of seed germination (Baskin et al., 2006).

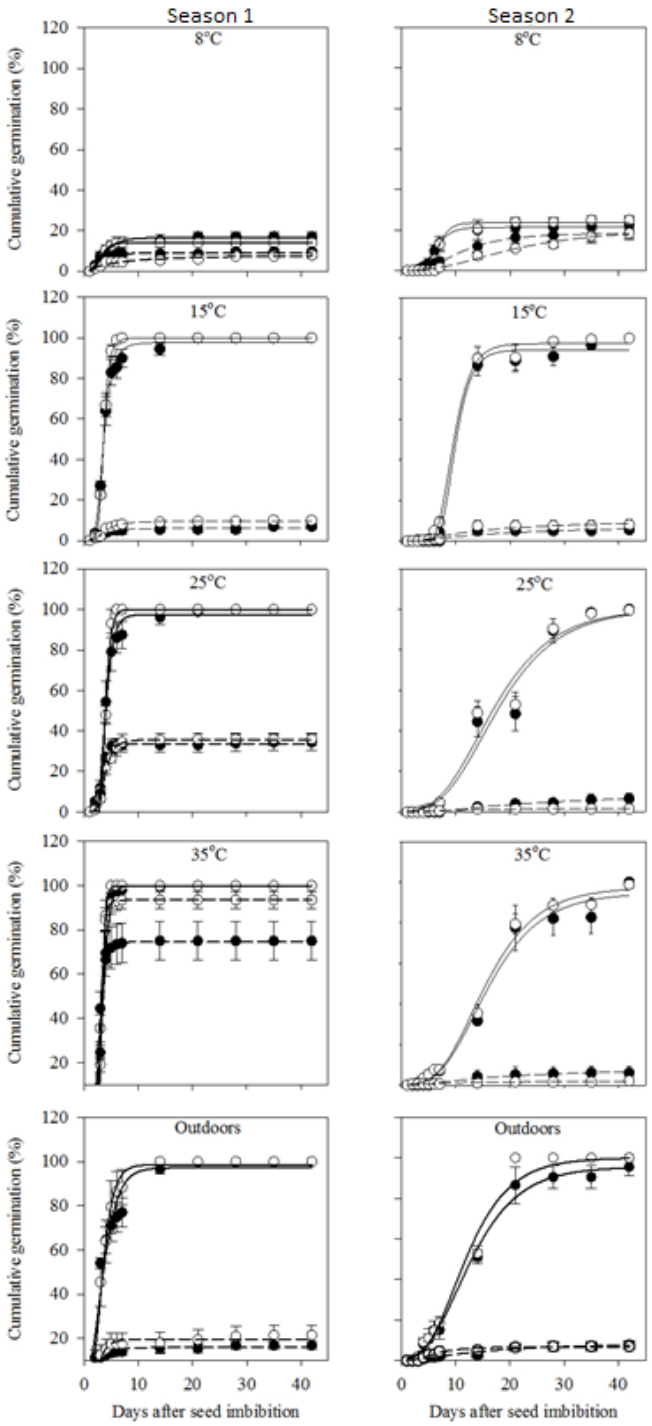

Figure 1. Cumulative germination pattern of 14 days after-ripened seeds of glyphosate-susceptible (S; ●) and -resistant (R; ○) *Echinochloa colona* phenotypes under different after-ripening temperatures for (a) Season 1 and (b) Season 2 over a period of 42 days after seed imbibition. The fitted model for fluctuating 12-h day/night treatment (---- solid lines) was a three-parameter Gompertz function, while a three-parameter Hill or exponential function was fitted to the constant darkness treatment (- - - dashed lines). Symbol bars indicate the standard error of mean (n = 4) for each after-ripening temperature regime, time and phenotype.

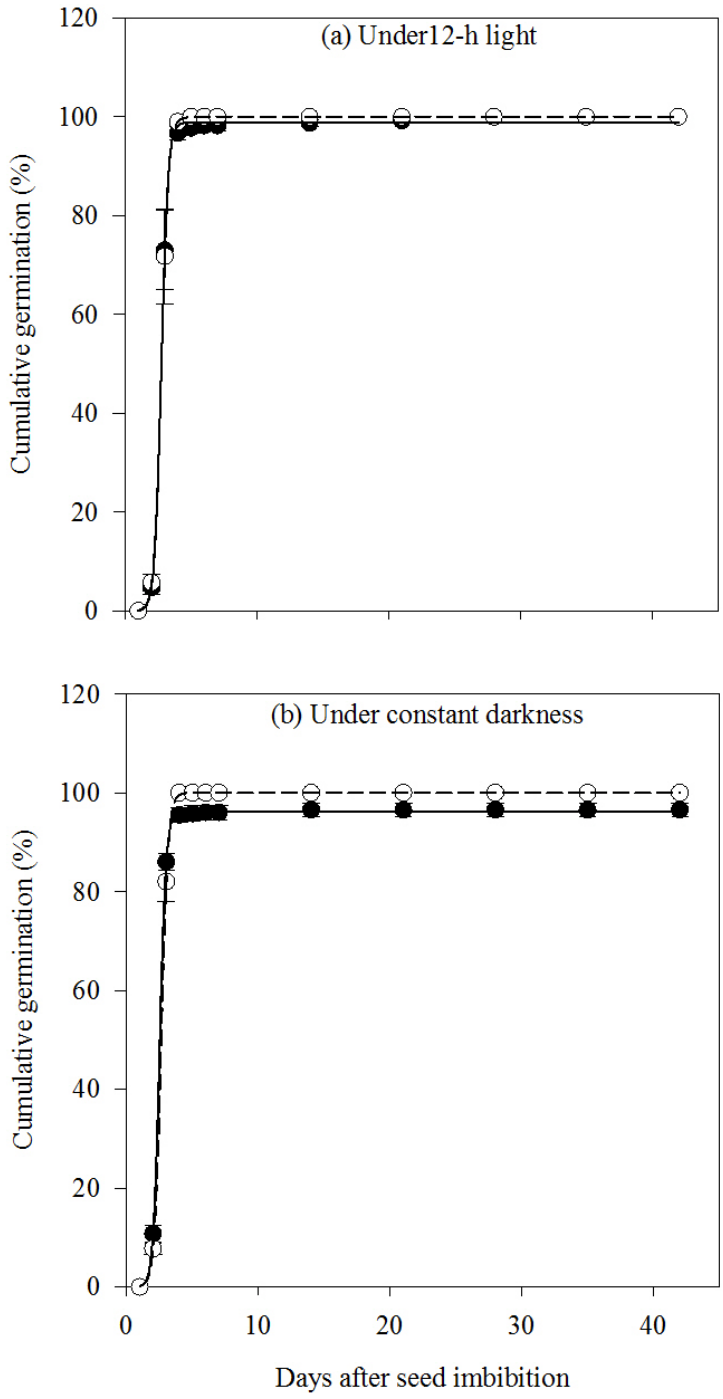

Figure 2. Germination pattern for non-dormant seeds of glyphosate-susceptible (S; ●) and -resistant (R; ○) *Echinochloa colona* over a period of 42 days after seed imbibition on 1% (w/v) agar solidified water. The germination test was conducted in a growth incubator at an alternating 30/20°C day/night temperature with (a) fluctuating 12-h light/dark period and (b) constant darkness. Symbol bars indicate the standard error of mean (n = 8) for each light regime, time and phenotype. Regression lines represent the fitted three-parameter sigmoidal regression model.

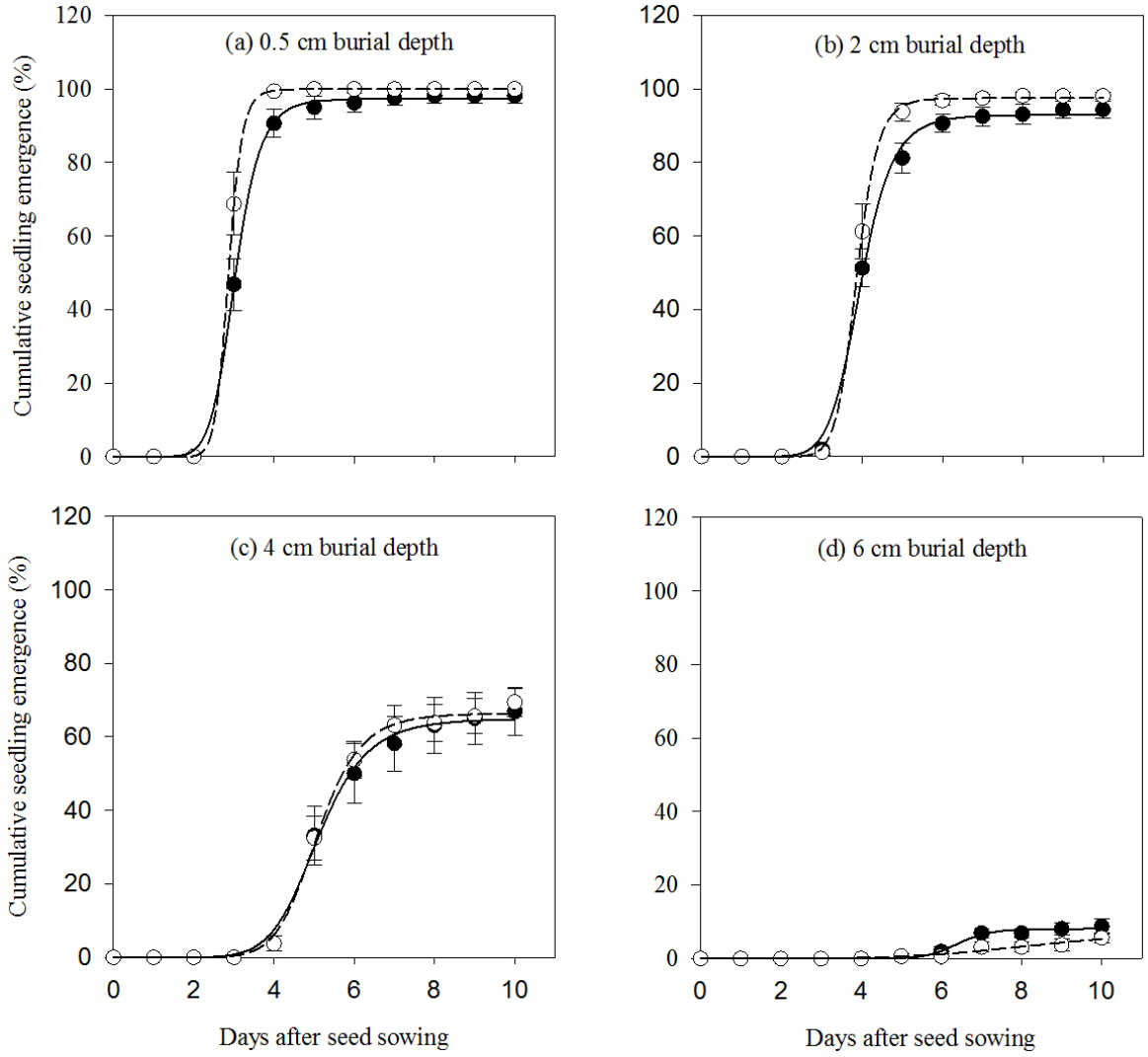

Figure 3. Cumulative seedling emergence for the seeds of glyphosate-susceptible (S; ●) and -resistant (R; ○) *Echinochloa colona* phenotypes sown at the depth of (a) 0.5 cm, (b) 2 cm, (c) 4 cm and (d) 6 cm from the soil surface. Symbol bars indicate the standard error of mean (n = 8) for each burial depth and phenotype. Regression lines represent the fitted three-parameter hill regression model.

By using plant materials that shared a similar genetic background, Délye et al. (2013) found that an acetyl-coenzyme A carboxylase (ACCase) resistance allele, Asn2041, had no significant effect on seed germination of *Alopecurus myosuroides* Huds., whereas the Gly2078 and Leu1781 resistance alleles showed accelerated germination compared with the susceptible genotype. Vila-Aiub et al. (2005) revealed a fitness cost expressed under darkness of the ACCase target-site Ile1781Leu mutation as emergence and fatal germination of herbicide resistant *L. rigidum* seeds were lower than genetically related seeds exhibiting a susceptible ACCase or metabolism-based cytochrome P450 resistance.

Conversely, some other studies have found that herbicide susceptibility is associated with better germination than the corresponding resistant plants in weed species such as triazine-susceptible *Brassica campestris* L. (Mapplebeck et al., 1982). A wealth of studies have shown that higher dormancy in some weed species is correlated with herbicide resistance and intensive cropping systems because the dormant seeds, by germinating late, avoid the period of selection pressure of non-selective herbicides (Gundel et

al., 2008; Owen et al., 2015). In this study, a lack of a germination-related fitness cost was observed in the R phenotype of *E. colona*. The results suggested that the glyphosate-resistant trait was unlikely to be involved in the expression of pleiotropic effects on the physiological and biochemical mechanisms leading to seed germination and seedling emergence.

Management of the *E. colona* seed bank

Management of herbicide-resistant weeds has been widely discussed elsewhere (Evans et al., 2016; Norsworthy et al., 2012). Nevertheless, specific measures based on seed germination ecology are scarce. The similar seed germination ecology between the S and R phenotypes observed in the present study implied that any weed control measure, targeting the weed seed bank or young seedlings, can be used with equal effectiveness on each phenotype and thus enhance the efficiency of farming operations to manage this weed. Effective control of *E. colona* has also been found by pre-emergent herbicides atrazine and metolachlor in sorghum planting (Wu et al., 2004).

Rapid loss of seed dormancy and higher seedling emergence at shallow soil depths coupled with greater fatal germination at deeper burial depths revealed that *E. colona* S and R phenotypes exhibited a short seedbank life. This conformed to Uremis and Uygur (2005), and Walker et al. (2010) who found similar results for this species, probably due to the scarcity of seed carbohydrate reserves in small-seeded *E. colona* that restrict them from emerging from greater burial depths. Other studies have also shown that seedling emergence of *E. colona* was optimum at the shallow upper soil horizon in a range of 0 to 2 cm burial depths (Chauhan and Johnson, 2009; Walker et al., 2010; Wu et al., 2004). In Australia, flushes of *E. colona* in the field have been observed in the wetter and warmer months of October to March indicating that emergence of this species is highly correlated with temperature and the availability of soil moisture (Walker et al., 2010; Wu et al., 2004).

Seeds and seedlings have been targeted in designing weed control strategies due to their high vulnerability and ease of control at this stage (Ghersa et al., 2000). From the perspective of *E. colona* management, minimal tillage with shallow burial may provide favourable conditions to induce faster dormancy breakdown and emergence of *E. colona*. Once the seeds have germinated or seedlings have established, the control of young *E. colona* plants could be achieved with herbicides or other treatments, thus reducing the seedbank in the field.

Harvest weed seed control techniques, which are aimed at minimising replenishment of the weed seed bank (Walsh and Powles, 2014), appear to have little potential to control *E. colona* at crop harvest as they shatter and drop their seeds when ripe. The no till cropping system would prevent deeper buried seeds being returned to the soil surface. No till systems may also delay seedling emergence, which would provide an advantage in the early stage of crop establishment. It was reported that late emergence of *Echinochloa* spp. due to rice canopy closure has successfully suppressed this weed (Gibson et al., 2002). Thus, cultural practices such as narrow planting rows, high crop seeding rate and the use of competitive crop cultivars could be implemented, wherever appropriate, to suppress late-emerging *E. colona* seedlings and thus reduce the competitiveness of this weed.

CONCLUSIONS

Similar seed dormancy release, germination and seedling emergence patterns between the S and R phenotypes of *E. colona* demonstrated that at this stage or ontogeny, a physiological fitness cost was not evident in the R phenotype. As no pleiotropic effect could be found between glyphosate resistance traits and the ecology of the R-*E. colona* seed, it remains a challenge to design specific management recommendations to target and control the R phenotype. However, a diverse integrated management approach should aim to integrate cultural, mechanical and chemical tactics to reduce the sole reliance on glyphosate for *E. colona* control and provide effective long-term management.

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