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Effect of temperature and light on germination behavior of PSII inhibiting herbicide resistant and susceptible junglerice (*Echinochloa colona*) populations

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Abstract

Repeated use of triazine herbicides have resulted in evolution of triazine resistant junglerice (*Echinochloa colona*) populations in southwestern Iran. Experiments were conducted under controlled conditions to investigate the effect of temperature and light on germination of two triazine-resistant (R2 and R3) and one susceptible (S) junglerice populations. The germination response was evaluated at nine constant temperatures of 10, 15, 20, 25, 30, 35, 40, 45 and 50 °C and two alternating temperature regimes of 20/10 and 30/20 °C. For each population, the cumulative germination over time was modeled using a Weibull model. The three-piece segmented model was also used to describe the cardinal temperatures for the germination rate of junglerice populations. The R2 population was greatest germination in both temperature regimes. The time taken to reach 50% germination (t_{50}) decreased with the increasing temperature in all populations. Based on the three-piece segmented model outputs, the base, the lower optimum, the upper optimum and the maximum temperatures for the germination and 9.89, 22.12, 34.05 and 45.25 °C for S population. Also, these values were 9.77, 20.48, 35.73 and 49.90 °C for R2 population and 9.89, 22.14, 30.33 and 46.08 °C for R3 population. Germination of all populations was stimulated after the transfer of seeds kept in darkness to the light. We suppose resistance to triazine and triazinone herbicides not only did not have created negative effect on germination characteristics of R-biotype but also had created competitive advantage in its germination behavior.

Keywords: Cardinal temperatures, germination rate, Weibull model, three-piece segmented model. **Abbreviations:** Triazine herbicide_The herbicide group (C3) that inhibit photosynthesis in photosystem II; R2 and R3_The resistant populatios; S_the susceptible population.

Introduction

Junglerice (Echinochloa colona) is an annual grass and genetically self-pollinated species that is widespread across sugarcane-growing regions in southwest of Iran. This summer-germinating annual grass weed is often present at high densities and has recently evolved resistance to PSII inhibiting herbicides (Elahifard et al., 2013). For more than 30 years, herbicides have been the principal means for weed control in sugarcane fields. Consequently, monoculture with repeated use of herbicide families with same mechanism of action has resulted in the evolution of populations resistant to herbicides (Devine and Shukla, 2000 and Gadamski et al., 2000). Weed control programs in sugarcane fields are regularly applied at three different stages in time during summer cropping system. First, pre-emergence control with herbigation (the first irrigation water) on September; Second, early post emergence control on October that eliminates weed seedlings arising from less dormant seeds; Third, post emergent selective herbicides and mechanical operation (hilling up) are applied to those weed seedlings which germinate from the remaining dormant seeds in seed bank, after the sugarcane is grown about 50 cm tall on February until June. This farming practice, when used annually, results in a strong selection pressure for herbicide resistance in weed seedlings emerging after the crop is planted. For the in-crop selective herbicides, resistance can develop in as few as 4 years (Owen et al., 2010). Managing herbicide-resistant weeds requires good understanding junglerice biology, specially the timing of seedling emergence. Environmental factors such as temperature, soil solution osmotic potential, solution pH, light quality, management practices and seed location in the soil seed bank, affect weed seed germination emergence (Northworthy and Oliveira, 2006). and Suggestions have occasionally been made in the literature of a link between herbicide resistance and seed dormancy. Of particular relevance to this study is the measurement of differences in seed germination between herbicidesusceptible and herbicide-resistant individuals and populations of junglerice. Fitness has been identified as an important factor influencing the evolution and dynamics of herbicide resistance (Park and Mallory-Smith, 2005). There are two possible sources of reduced fitness: the gene modification that confers herbicide resistance results in a protein with reduced efficiency; or deleterious genes have became associated with the resistant individuals as a result of the founder effect during selection for resistance (Purba et al., 1996). The resistance of weed populations to the triazine herbicides, a chemical group that inhibits photosystem II, with a few exceptions (Schonfeld et al., 1987), results in the reduced vigour, growth and competitive ability when compared with susceptible populations in the absence of herbicide (Soltani et al., 2008). Differential fitness of resistant and susceptible weed species is an important factor for predicting the evolution of herbicide resistance (Wiederholt and Stoltenberg, 1996). Fitness differences between herbicide-resistant and herbicide-susceptible populations are usually inferred from comparisons of relative plant vigor, productivity, or competitiveness, as measured using specific characteristics including: seed dormancy, flowering date, seed production, above ground biomass, and other factors that affect the likelihood of the survivorship and fecundity of the species (Massinga et al., 2005). When the resistance mutation is associated with a sacrifice in plant fitness, the evolution of herbicide resistance in a population is expected to be slower (Wiederholt and Stoltenberg, 1996). For successful cultivation of sugarcane, sustainable management of the resistant biotype of junglerice is important. To date, no information has been reported on the germination and emergence of the resistant and susceptible populations of junglerice. Understanding the biology of the species is imperative to achieving effective control of the Rbiotype of junglerice. The objectives of this research were to determine germination ability of junglerice populations resistant to triazine herbicides compared with a susceptible biotype (S). We hypothesized that (1) herbicide resistance and dormancy are not associated (2) Susceptible populations have been associated with greater dormancy than resistant populations.

Results and discussion

Effect of temperature and light on germination

For all populations, germination was observed at temperature range 10-45 °C. However, no germination was observed at the highest constant temperature (50 °C) (Fig. 1). Seed germination for the - R and -S populations exceeded 94% at 20, 25 and 30 °C (Fig. 1). At lower and higher temperature (10 and 45 °C), however, germination was significantly greater in the R2 biotype than others (Fig. 1). The ability to germinate over the range enables this species/population to emerge throughout the year in cropping system. Also, comparing germination of populations in darkness at three temperatures (25, 30 and 35 °C) showed that increasing temperature could not replace light requirement in seeds (Fig. 2). So, germination percentages for all populations varied significantly at three temperatures in both conditions (Fig. 2). When not- germinated seed in darkness were transferred to light, demonstrating significant effect of light on seed germination. So that, germination percentage of the S, R2 and R3 populations from 58, 60 and 42 at 25 °C, 58, 67 and 30.75 at 30 °C and 20, 25 and 11 at 35 °C varied to 91, 78 and 66, 90, 98 and 75 and 93, 95 and 68% (Fig. 3). As well as, germination percentage of the S population at 25 °C, the S and R2 populations at 30 °C and the R2 and R3 populations at 35 °C, were not significantly different in light and exposing light after darkness (Fig. 3). These results disagree with Darmeny and Pernes (1989) who reported a lower germination percentage for triazine resistant Setaria italica than susceptible biotype. Also, Park and Mallory-Smith (2005) stated triazine resistant Bromus tectorum produced less shoot dry weight, leaf area and seed than susceptible biotype. But, these results agree with Schonfeld et al. (1987) who reported a triazine-resistant biotype of Phalaris paradoxa that was superior to a triazine -susceptible biotype in seed germinability and seedling emergence. Differences in

germination percentage between triazine -resistant and susceptible junglerice populations disagree with most previous studies. As shown in Fig 4. cumulative germination of the S, R2 and R3 populations at 6, 7 and 7 d were greatest in the 30/20 °C temperature regime with light/dark (86.25, 100 and 85.20%) (P \leq 0.01). Whilst, the values for the populations at 6, 8 and 8 d were greatest in the 20/10 °C temperature regime with light/dark (78, 100 and 32%) (P≤ 0.01). The process was longer in darkness than light/dark at 20/10 °C exception the R3 population. Also, maximum cumulative germination (40, 41 and 17%) at 30/20 °C temperature regime with darkness were lesser than those of in light/dark, but the time to reach it (4, 7 and 4 d) were lesser than from those of the light/dark condition. The t₀, t₅₀ and G_{max} values for all populations varied significantly in the two conditions (Fig. 4 and Table 1). For instance, t₀ for three populations ranged from -0.27 to 2.49 at 20/10 °C in light/dark, in contrast to the range of 3.55 to 3.98 at 20/10 °C in darkness. Whereas, the values were lower than those at $30/20\ ^\circ C$ in both conditions. The G_{max} ranges for the populations were 32.13 to 99.72 and 14.18 to 55.18 at 20/10 °C in light/dark and darkness, respectively. Also, it ranged from 86.39 to 100.61 and 17.01 to 41.19 at 30/20 °C in light/dark and darkness, respectively. The G_{max} value was the highest in the R2 population in both temperatures. At 20/10 °C day/night, the t_{50} value was about 4 days for all population that was lower than it in darkness. At 30/20 C day/night, also, it was 2 days for all population that was greater than it in darkness. It would be noticed that with increasing temperature from 20/10 to 30/20 °C, seed germination significantly increased for the S population in darkness. It indicates increasing temperature, may be, have been replaced with light requirement in this population. Hence, reducing dependence of resistant biotype on light for germinating at high temperature is one of its strengths in the tropics. Further, a proportion of the seed was capable of germinating regardless of light conditions, which, when combined with broad adaptation to temperature, would provide this weed with many opportunities to establish and reproduce. In a similar experiment, germination ryegrass resistant phenotype (target site based resistance) compared with other resistant plants (non target site based resistance) and susceptible was reduced in the darkness, indicating light requirement of R biotype was increased (Vila-Aiub et al., 2005).

Cardinal temperatures

Response of germination rate of junglerice to temperature was described using three-piece segmented model (Fig. 5). Based on the model outputs, base, lower optimum, upper optimum and maximum temperatures were 9.65, 22.12, 34.05 and 45.25 °C for the S population. Also, these values were 9.77, 20.48, 35.73 and 49.90 °C for the R2 population and 9.89, 22.14, 30.33 and 46.08 °C for the R3 population. Based on the model estimation, seeds of the R2 population enable to germinating in higher temperatures than other populations. Although, temperature base of the populations were alike. Also, the populations were similar in the minimum number of days for germination at the optimum temperature (Table 2). Furthermore, the optimum temperature range was greatest (15.25 °C) for the R2 population in contrast to the S and R3 that were 11.93 and 8.19 °C, respectively (Table 2). It would be noticed that the effects of temperature on plant development are the basis for models used to predict the timing of germination. The cardinal temperatures describe the range of this environmental variable over which seeds of a particular species can germinate (Derakhshan et al. 2014).

Temperature (°C) 20/10 °C 30/20 °C Day/night photoperiod Darkness Day/night photoperiod Darkness Population R2 R3 R2 R3 R2 R3 S R2 Parameter S S S R3 -0.27 (0.82) 2.26 (0.11) 2.49 (0.12) 3.55 (0.64) 3.88 (0.03) 3.98 (0.01) 1.00 (0.01) 1.97 (0.03) 1.99 (0.01) 0.99 (0.04) 1.00 0.9992 t_0

14.18

(0.20)

4.91 (0.08)

1.23 (0.21)

86.39 (0.08)

1.89 (0.01)

1.15(0.01)

100.61

(0.61)

1.98 (0.02)

0.29 (0.08)

(0.0001)

41.19 (0.18)

1.51 (0.03)

0.73(0.04)

40.06

(0.10)

1.86 (0.02)

1.37 (0.07)

87.83 (2.77)

2.01 (0.01)

0.25(0.09)

(0.01)

17.01

(0.01)

1.63 (0.01)

1.24 (0.03)

Table 1. Estimated parameters for cumulative germination percentage of triazine and triazinone -resistant (R2 and R3) and –susceptible (S) *Echinochloa colona* seeds in response to alternating temperature (20/10 and 30/20 °C) at different conditions (14/8-h day/night photoperiod and darkness) using Weibull model.

Abbreviations: to, time between the beginning of the incubation and the first germination; G_{max}, maximum germination percentage; t50, time to 50% germination; b, shape parameter.

19.80

(0.21)

5.48 (0.06)

2.58 (0.90)

55.18 (0.23)

4.14 (0.02)

0.62 (0.06)

G_{max}

 t_{50}

b

78.04

(0.21)

4.01 (0.02)

5.64 (1.11)

99.72

(0.19)

3.96 (0.01)

2.71(0.19)

32.13

(0.17)

4.35 (0.03)

1.49 (0.12)

Table 2. Estimated parameters for germination rate of triazine and triazinone -resistant (R2 and R3) and –susceptible (S) *Echinochloa colona* seeds in response to different constant temperatures using three-piece parameter model.

	Population		
Parameter	S	R2	R3
T _b	9.65 (1.27)	9.77 (0.27)	9.89 (1.27)
T _{o1}	22.12 (1.81)	20.48 (0.33)	22.14 (1.87)
T _{o2}	34.05 (1.48)	35.73 (0.59)	30.33 (2.91)
T _c	45.25 (1.12)	49.90 (0.35)	46.08 (1.83)
f_o	0.11 (0.01)	0.10 (0.002)	0.11 (0.01)

Abbreviations: T, temperature; T_o, base temperature; T_o, optimum temperature; T_o, lower optimum temperature; T_o, upper optimum temperature; T_o, maximum temperature; T_o, minimum number of days for germination at the optimum Temperature.



Fig 1. Effect of constant temperature on germination of triazine and triazinone -resistant (R) and –susceptible (S) *Echinochloa colona* seeds with 14/8-h day/night photoperiod for 3 weeks. Vertical bars represent the standard errors of the mean values. Means that do not share a letter are significantly different ($P \le 0.05$).



Fig 2. Effect of temperature and light condition on germination of triazine and triazinone -resistant (R) and –susceptible (S) *Echinochloa colona* seeds for 3 weeks. L and D represent 14/8-h day/night photoperiod and continuous darkness, respectively. Vertical bars represent the standard errors of the mean values. Means that do not share a letter are significantly different ($P \le 0.05$).

Materials and Methods

Seed collection

Sugarcane fields were surveyed in the 2010 growing season, with junglerice seeds collected over a period of 2 month, July to August 2010. In total 20 sugarcane fields were visited across agronomic zone in the Karun Agro- Industry Inc., Khuzestan province, Iran. All samples were stored in paper bags in a room before being moved to the laboratory at 25 °C.

Herbicide resistance testing

Herbicide resistance status was determined by treating 3- to 4-leaf stage seedling of suspected junglerice populations with two herbicides, using the upper recommended rate and assessing plant mortality 28 days after treatment. Detailed greenhouse assaying is described in Elahifard et al. 2013.

Laboratory studies

Laboratory studies were conducted to evaluate germination ability and dormancy behavior in three populations, one susceptible (S) and two triazine resistant (R2 and R3) junglerice in response to temperature regimes. Germination of R- and S-seeds was tested on filter paper moistened with distilled water in Petri dishes, 25 seeds per dish, with 4 replications for each population. The dishes were incubated in germinator (10, 15, 20, 25, 30, 35, 40, 45 and 50 °C constant temperatures with light/dark) and (20/10 and 30/20 °C alternating day/night temperature with light/dark). Seed coats were removed by washing with H_2SO_4 (95-97%) for 10 min. Germination was counted daily for 2 and 3 weeks for alternating and constant temperature regimes, removing the germinated seeds after each counting. Germination was determined by visible radicle protrusion for any seed. To evaluate the necessity of Light for germination populations, after the end of germination experiment in darkness, not-germinated seeds exposed to in light/dark.

Cardinal temperatures determination

For each population, the three-piece segmented function (Eqn 1) was used to quantify the response of germination rate to constant temperatures (°C) according to Piper et al. (1996):. $f(T)=(T-T_b)/(T_{o1}-T_b)$ if $T_b < T < T_{o1}$

(1)	
$f(T) = (T_c - T) / (T_c - T_{o2})$	if $T_{o2} < T < T_c$
f(T)=1	if $T_{o1} \le T \le T_{o2}$
f(T)=0	if $T \le T_b$ or $T \ge T_c$



Fig 3. Effect of temperature and light condition on germination of triazine and triazinone -resistant (R) and -susceptible (S) *Echinochloa colona* seeds for 2 weeks. L and D-L represent 14/8-h day/night photoperiod and exposed to the light after darkness, respectively. Vertical bars represent the standard errors of the mean values; Means that do not share a letter are significantly different ($P \le 0.05$).



Fig 4. Predicted (–) vs. observed (\bullet , \bigtriangledown , \blacksquare) cumulative germination percentage of triazine and triazinone -resistant (R2 and R3) and -susceptible (S) *Echinochloa colona* seeds in response to alternating temperature (20/10 (a, b) and 30/20 (c, d) °C) at different conditions (14/8-h day/night photoperiod (a, d) and darkness (b, c)) using Weibull model. Vertical bars represent the standard errors of the mean values.



Fig 5. Predicted (lines) germination rate of *Echinochloa colona* seeds at different constant temperatures using three –piece segmented model.

Where T is temperature, T_b is base temperature, T_o is optimum temperature, T_{o1} is lower optimum temperature, T_{o2} is upper optimum temperature, T_c is maximum temperature. Also, the cumulative germination percentage (G) at alternating temperatures was plotted against time (in days). From this curve, the time to 50% germination (t_{50}) was determined by fitting a Weibull equation (Eqn 2) to cumulative germination percentage (G) against time (t, in days) according to Guillemin et al. (2012): G=0 if t<t_0

G= m $[1-e^{-\ln (2)} \times (t-t_0 / t_0^{-t_0})^b]$, if t>t0 (2)

Where m is the maximum germination percentage, t_0 is the time between the beginning of the incubation and the first germination and b is shape parameter.

Statistical analysis

All experiments were conducted with the use of a completely randomized design (CRD) with four replicates. Data analysis was carried out by Minitab software (Minitab Inc, Version 16.2.4). Significant differences among treatments were identified with the use of Duncan test ($P \leq 0.05$). Also, SigmaPlot software (SigmaPlot 2008 for Windows, Version 11.0, SPSS Inc.) was used to calibrate the models (Weibull and three -piece segmented) via an iterative optimization method.

Conclusion

As shown, some resistant population such as the R2 could germinate more and faster than others. The R2 population was highest in germination rate and uniformity at both temperature regimes. Therefore the fitness cost of resistant population in the comparative experiment was not shown. Most researchers concluded reduction in fitness of the resistant biotype is probably because of the altered D1 protein. The results of previous experiment revealed nucleotide changes (A to G) at position 232 conferred amino acid substitution from serine to glycine at residue 264 in the R- populations. Although the R -populations were heterozygous at this position. Thus it can be concluded resistance to triazine herbicides depends on the specific resistant allele(s) conferring biotype traits. As germination of junglerice occur in broad range of temperature, this species is likely to invade other areas especially tropics and subtropics. The results of this study identify some of the factors that allow junglerice to be a widespread and problematic weed in the tropics and subtropics and this information may contribute to its control. Since, the R- population is able to germinate earlier and faster than populations we could manage it by stale seed bed. So this method can be applied towards discharging the seed bank of this population.

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