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Effect of Environmental Factors on Germination and Emergence of Aryloxyphenoxy Propanoate Herbicide-Resistant and -Susceptible Asia Minor Bluegrass (*Polypogon fugax*)

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The influence of environmental factors on germination and emergence of aryloxyphenoxy propanoate herbicide-resistant (AR) and -susceptible (AS) Asia Minor bluegrass were studied in laboratory and greenhouse experiments. Seeds were collected from AR and AS plants cultivated in separate greenhouses under the same environmental conditions. The results revealed that optimum temperatures for the germination of AS biotype were 10 to 25 C or alternating temperature of 15/5 to 30/20 C and light was not necessary. However, maximum germination occurred at 10 C or 15/5 C, and no germination occurred above 15 C or 25/15 C for the AR biotype. The AS Asia Minor bluegrass was consistently more tolerant to environmental stress, as evidenced by their greater germination at same pH value, osmotic potential, and NaCl concentration at 15/5 C compared to the AR biotype. Higher emergence rates were obtained when seeds were sown on the surface of soil for both biotypes. Emergence percentage of the AR biotype was below 14% when buried, whereas the AS biotype had 20% emergence at 2.5 cm burial depth. It is concluded that several environmental factors affect the germination of Asia Minor bluegrass, and the AS biotype showed higher germination percentage and a wider adaptive range under same treatments compared with the AR biotype. Due to the reduced emergence at depth, deep tillage could be an effective management to reduce AR Asia Minor bluegrass infestation in the following crop.

Nomenclature: Asia Minor bluegrass, *Polypogon fugax* Nees ex Steud.
Key words: Osmotic potential, pH, salt stress, seed burial depth, temperature.

Asia Minor bluegrass is a common annual grass weed in winter crops, such as wheat (*Triticum aestivum* L.) and rapeseed (*Brassica napus* L.), as well as in wasteland and along roadsides across China (Li 1998). It used to be a sporadic species, whereas shortawn foxtail (*Alopecurus aequalis* Sobol.), Japanese foxtail (*Alopecurus japonicus* Steud.), and American sloughgrass [*Beckmannia syzigachne* (Steud.) Fernald] were the main weeds of winter crops (Li et al. 2013; Tang et al. 2012). It has been reported that agronomic factors such as crop rotation, tillage, and herbicide use, affect weed communities (Hume et al. 1991; Shrestha et al. 2002). As a result of increased acreage under reduced tillage and stubble retention, relatively restricted herbicide regimes (tribenuron-methyl and fenoxaprop-p-ethyl), and limited crop rotations during the last two decades, the weed community and predominant species have changed, and Asia Minor bluegrass has become a problematic weed in wheat/rapeseed fields rotated with rice (Chen et al. 2013; He et al. 2004; Wang and Qiang 2002). This weed strongly competes with these winter crops and can reduce wheat yield by up to 40% (Zhang 1993).

Germination is one of the most critical stages in weed establishment. Successful establishment of weeds depends heavily on the weeds' ability to germinate and emerge under a wide range of environmental conditions. In general, the pattern of emergence results from interactions between numerous internal and external factors. Several internal factors affect seed germination, and include seed vitality, maturation, dormancy, and genotype (Bewley and Black 1994). Environmental factors, such as temperature, light, pH value, soil moisture, and seed burial depth, are also known to affect seed germination and emergence (Chachalis and Reddy 2000; Chauhan et al. 2006a; Koger et al. 2004; Taylorson 1987).

Aryloxyphenoxy propanoate herbicide-resistant (AR) Asia Minor bluegrass has been reported in Qingshen county of Sichuan province in China, where failure of acetyl coenzyme A carboxylase (ACCase) inhibitors to control this weed was observed after several years of successful control.

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(Tang et al. 2014). A previous study showed that the AR biotype exhibited acclerated phenological development compared to the aryloxyphenoxy propanoate herbicide-susceptible (AS) biotype. In the AR biotype, head emergence, flowering, and seed maturation were earlier than in the AS biotype (W. Tang et al. unpublished data).

To date, no detailed study has been conducted specifically to investigate the germination and emergence biology of Asia Minor bluegrass. Understanding the biology of AR and AS is useful in estimating the potential spread to new croplands and achieving effective control of the resistant biotype of Asia Minor bluegrass. The objective of this research was to: (1) determine the effects of temperature, pH, osmotic and salt stress, and burial depth on seed germination and seedling emergence of Asia Minor bluegrass; and (2) compare the response of the AR biotype to these environmental factors with the AS biotype.

Materials and Methods

Seed Preparation. An Asia Minor bluegrass accession collected in 2011 in Qingshen county of Sichuan province, China (29.54°N, 103.48°E), which was previously analyzed by ACCase genotyping (Ile-2041-Asn substitution of acetyl-coenzyme A carboxylase; Tang et al. 2014), and an AS biotype from a noncultivated area (about 2.5 km from the field where the AS biotype was collected), were separately cultivated in greenhouse at the Zhejiang Chemical Industry Research Institute (ZCIRI, 30.15°N; 120.03°E), Hangzhou, Zhejiang province, China. Seeds collected from plants of the two biotypes in May 2013 were used to determine the differences in seed germination and seedling emergence of the AR and AS Asia Minor bluegrass. The collected seeds were air-dried and stored in paper bags at 4 C for 3 mo to break dormancy, and then stored at room temperature (20 ± 5 C) until used. The mature seed color was yellow, and the 1,000-seed weight (with bran) was 145 and 150 mg for the AS and AR Asia Minor bluegrass biotypes, respectively. Experiments were conducted at the ZCIRI in January and May 2014.

General Germination Test. Fifty seeds of the AR and AS biotypes of Asia Minor bluegrass were evenly placed on two layers of Whatman No.1 filter papers in 9-cm-diam petri dishes. Filter papers were moistened with 5 ml distilled water or test solution. Petri dishes were sealed with Parafilm (American National Can, Greenwich, CT) to reduce loss of water, and were placed in an incubator (RTOP-310D, Zhejiang Top Instrument Co., Ltd., Hangzhou, China) at 15/5 C with a 12-h night cycle. Fluorescent lamps were used to produce a photosynthetic photon flux density of 3,000 Lux unless otherwise specified. Germination was determined by visible radicle protrusion 15 d after the start of the experiments.

Effect of Temperature. Seeds of the AR and AS biotypes were incubated under constant (5, 10, 15, 20, 25, 30 C) or fluctuating day/night temperatures (15/5, 20/10, 25/15, 30/20, 35/25 C). Photoperiod was set at 12 h to coincide with the high-temperature period.

Effect of Light. Seed germination was studied under 12/12, 0/24, 24/0 h light/dark regimes per 24-h cycle at 15/5 C. Dishes assigned to the dark treatment were wrapped in a double layer of aluminum foil, and other treatments were maintained uncovered to allow continuous light exposure (3,000 Lux).

Effect of pH. Germination as affected by pH was studied using buffer solutions of pH 4 to 10 prepared according to Chachalis and Reddy (2000): a 2 mM potassium hydrogen phthalate buffer solution was adjusted to pH 4 with 1 N HCl; a 2 mM solution of 2-(*N*-morpholine) ethanesulfonic acid was adjusted to pH 5 or 6 with 1 N NaOH; a 2 mM solution of *N*-(2-hydroxymethyl) piperazine-*N*′-(2-ethanesulfonic acid) was adjusted to pH 7 or 8 with 1 N NaOH; and a pH 9 or 10 buffer was prepared with 2 mM tricine [*N*-tris (hydroxymethyl) methylglycine] and adjusted with 1 N NaOH. Unbuffered distilled water (pH 7.2) was used as a control. The petri dishes were incubated at 15/5 C day/light with a 12-h photoperiod (provided optimum germination according to the temperature and light experiment) as described in the general germination test.

Effect of Osmotic and Salt Stress. Aqueous solutions with osmotic potential of 0, −0.1, −0.2, −0.3, −0.4, −0.5, −0.6, and− 0.7 MPa were prepared by dissolving 0, 62.6, 99.7, 128.9, 153.9, 176.0, 196.0, and 214.5 g polyethylene glycol 6000 in 1 L of distilled water, respectively (Michael and Kaufman 1973). Sodium chloride solutions of 0, 10, 20, 40, 80, and 160 mM were prepared to investigate salt stress on seed germination. Petri dishes were incubated as described in the pH experiment.

Effect of Burial Depth. Fifty seeds of AR and AS Asia Minor bluegrass were planted in soil in 7.5-cm-diam plastic pots at 0, 0.5, 1.0, 1.5, 2.0, and 2.5 cm depth, respectively. The soil used was a sterile potting medium (mixed vegetable garden soil/cover soil, 4 : 1, v/v) with pH 6.29 and 13.71% organic matter. Greenhouse temperatures were 15 ± 2 C during the day and 5 ± 3 C during the night with a 12-h photoperiod (150 to 500 μmol photons $m^{-2}s^{-1}$). The soil was maintained wet throughout the experiment by adding water to saucers placed under the pots. Seedlings were considered emerged when the cotyledon could be visually discerned, and emerged seedlings were counted 30 d after planting.

Statistical Analysis. A randomized complete block design was used in all experiments with three replications, and each experiment was repeated once. The data of the repeat experiments were pooled because no experiment by treatment interaction was revealed. All data met normality conditions. Nonlinear regression analysis was used to determine the effect of pH, salinity, and osmotic stress on seed germination, and seed burial depth on seedling emergence. Data obtained from temperature and photoperiod experiments were subjected to ANOVA with the use of SPSS software (version 13.0, SPSS Inc., Chicago, IL). Mean comparison was performed using least significant difference (LSD) post hoc tests, where the overall differences were significant ($P \leq 0.05$).

Results and Discussion

It has been suggested that differences in fitness, such as germination and emergence behavior, between the resistant and susceptible seeds might be confounded by differences in response to both biotic and abiotic environmental factors among populations and several susceptible and resistant biotypes should be included for comparison studies of fitness characteristics (Fenner 1991; Jasieniuk et al. 1996). In the present study, only one resistant biotype and one reference susceptible biotype were compared. In order to minimize differences in environmental factors, the AR and AS biotypes of Asia Minor bluegrass were collected from adjacent areas with similar growing conditions and then seeds of the two biotypes were grown for one generation in the greenhouse at the same temperature and light regimes.

Temperature. Under constant temperature conditions, the AS biotype of Asia Minor bluegrass germinated over a wider range of temperatures compared to the AR biotype. The highest germination (93%) was observed at 10 C (Table 1), and germination decreased to 82 and 67% at 20 and 25 C, respectively. The AR biotype only germinated at 10 and 15 C, with very low germination of 9 and 3%, respectively. The AS biotype could not germinate at temperatures ≤ 5 or ≥ 30 C. The optimum germination (78%) of the AR biotype of Asia Minor bluegrass occurred at fluctuating day/night temperature 15/5 C and then declined to 45% at 20/10 C and 2% at 25/15 C. No germination occurred at 10/0 C (data not shown) or 30/20 C and above. Germination of the AS biotype occurred over a wide range of fluctuating temperatures (15/5 C to 30/20 C) with germination of 90 to 97%, which was significantly ($P \leq 0.05$) higher than the AR biotype. The results of the fluctuating day/night temperature experiment is in agreement with the finding of Peng et al. (2011) that higher germination was observed at 20/15 C than at 25/20 C for Asia Minor bluegrass.

The response of the AS biotype of Asia Minor bluegrass to various temperatures, were neatly dovetailed with its biological characteristics and distribution in China. High germination at a wide range of constant or fluctuating temperature could be significant in the distribution of Asia Minor bluegrass. In China, this weed mainly occurs in the north, south, and southwest regions, which have a mean temperature of 5 to 25 C from middle October to late December. Cold regions in northeast and northwest China have temperatures below zero after November, which is inhibitory to the germination of this weed. The Ile-1781-Leu ACCase mutation has been shown to be associated with a strong environmentally determined dormancy in seed (Vila-Aiub et al. 2005). The AR biotype exhibited lower percentage germination compared to the AS biotype at higher temperatures. This might be caused by the fitness cost of the Ile-2041-Asn mutation and warrants further investigation.

pH. Germination of Asia Minor bluegrass over the pH range followed a nonlinear response to increasing pH, with increasing germination between pH 4 and 7 and decreasing germination from pH 8 to 10 (Figure 1). Maximum germination (97 and

Table 1. Effect of constant and fluctuating day/night temperatures on germination of aryloxyphenoxy propanoate herbicide-resistant (AR) and -susceptible (AS) Asia Minor bluegrass seeds incubated with a 12-h photoperiod for 15 d.

Temperature	Germination[a] AR	Germination[a] AS	Temperature	Germination[a] AR	Germination[a] AS
C	%		C	%	
5	0 f	0 f	15/5	78 c	97 a
10	9 d	93 a	20/10	45 d	96 a
15	3 e	90 a	25/15	2 e	96 a
20	0 f	82 b	30/20	0 f	90 b
25	0 f	67 c	35/25	0 f	0 f
30	0 f	0 f			

[a] For each experiment, means followed by the same letter are not significantly different according to Fisher's protected LSD at $P \leq 0.05$.

78%) was observed at pH 7.2 (distilled water) for both AS and AR biotypes, respectively, which was significantly ($P \leq 0.05$) different from all other treatments. Over the pH range 6 to 8, seed germination of AS biotype was > 55%. The AR biotype had germination ≥ 48% at pH 6 and 7 and ≤ 36% below pH 6 or above 8.

These results indicate that Asia Minor bluegrass can germinate over a wide range of pH, similar to other weed species such as Italian ryegrass [*Lolium perenne* L. subsp. *multiforum* (Lam.) Husnot, Nandula et al. 2009] and American sloughgrass (Rao et al. 2008). In China, Asia Minor bluegrass mainly occurs in wheat/rapeseed fields of the Yangtze River Valley, in places such as Sichuan, Anhui, and Jiangsu provinces; this region had soil pH ranges from 4 to 9 (Cheng et al. 2000; Huang et al. 2002; Ye and Li 2003), levels to which Asia Minor bluegrass is well adapted.

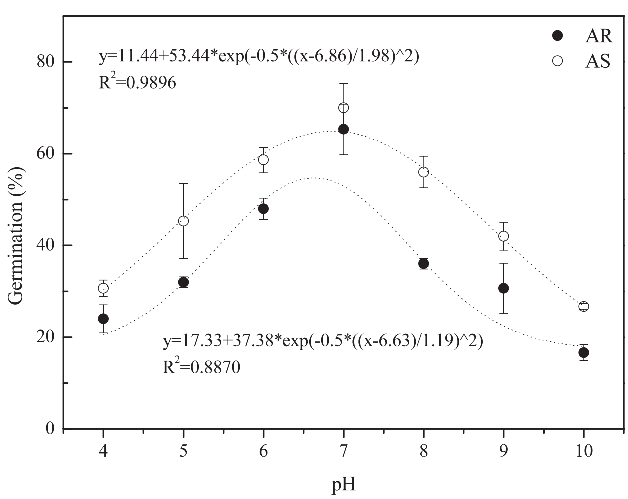

Figure 1. Effect of buffered pH on germination of aryloxyphenoxy propanoate herbicide-resistant (AR) and -susceptible (AS) Asia Minor bluegrass seeds at 15/5 C incubated with a 12-h photoperiod for 15 d. Vertical bars represent standard errors of the means.

Osmotic and Salt Stress. Two nonlinear exponential decay models were fitted to the germination percentages (%) obtained at different osmotic and salt stress levels for AR and AS Asia Minor bluegrass. Seed germination of both AR and AS biotypes of Asia Minor bluegrass decreased sharply as osmotic potential was reduced from 0 to −0.7 Mpa (Figure 2). No germination occurred at osmotic stress greater than −0.8 Mpa (data not shown). Germination was very low (< 27%) at an osmotic potential of −0.4 and −0.5 Mpa for the AR and AS biotypes, respectively. Germination of Asia Minor bluegrass occurred over a wide range of salt concentrations. Germination of the AS biotype was ≥ 85% when NaCl concentration was < 80 mM, which was significantly ($P \leq 0.05$) different from germination of the AR biotype (Figure 3). Seed germination decreased to 17 and 19% at 160 mM NaCl for AR and AS Asia Minor bluegrass, respectively, and no germination occurred at 320 mM NaCl (data not shown).

Osmotic and salt stress negatively affect germination of many weed species (Chachalis and Reddy 2000; Koger et al. 2004; Singh et al. 2012). Our results suggest that Asia Minor bluegrass has the ability to germinate under moderate soil salinity. Similar results were reported for annual sowthistle (*Sonchus oleraceus* L.; Chauhan et al. 2006b); tall morningglory [*Ipomoea purpurea* (L.) Roth; Singh et al. 2012], and horseweed [*Conyza canadensis* (L.) Cronq.; Nandula et al. 2006]. Asia Minor bluegrass seeds are sensitive to osmotic deficit and less sensitive to salinity, which is consistent with its hygrophilous nature, being distributed in wet soil and bottomland. Nandula et al. (2009) showed that a glyphosate-resistant Italian ryegrass biotype had a higher germination rate compared with the susceptible biotype. Conversely, our results indicate the AS Asia Minor bluegrass germinated

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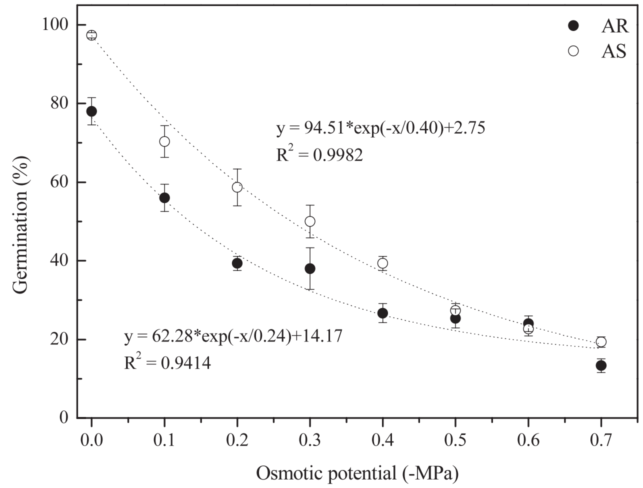

Figure 2. Effect of osmotic potential on germination of aryloxyphenoxy propanoate herbicide-resistant (AR) and -susceptible (AS) Asia Minor bluegrass seeds at 15/5 C incubated with a 12-h photoperiod for 15 d. Vertical bars represent standard errors of the means.

significantly better than the AR biotype at all osmotic and NaCl conditions.

Light and Burial Depth. Exposure to light had a significant effect on the germination of Asia Minor bluegrass (Table 2). When seeds were exposed to a 12-h photoperiod for 15 d, germination was 76% for the AR biotype, which was significantly higher ($P \leq 0.05$) than those in the dark. However, no seed germinated with a 24-h photoperiod. The AS biotype had similar seed germination (95 and 98%) under 0-h and 12-h photoperiods but this decreased to 30% with a 24-h photoperiod.

Table 2. Effect of photoperiod length on germination of aryloxyphenoxy propanoate herbicide-resistant (AR) and -susceptible (AS) Asia Minor bluegrass seeds at 15/5 C for 15 d.

	Germination[a]	
Photoperiod	AR	AS
h	%	
24	0 e	30 c
12	77 b	97 a
0	11 d	95 a

[a] Means followed by the same letter are not significantly different according to Fisher's protected LSD at $P \leq 0.05$.

Much lower seedling germination (< 15%) was recorded at a burial depth > 0.5 cm for the AR biotype, which was significantly ($P \leq 0.05$) different from emergence of the AS biotype. Seedling emergence of the AS biotype of Asia Minor bluegrass decreased slightly as the seed burial depth increased from 0 to 1.5 cm, but decreased sharply at depth > 2.0 cm ($y = 92.01 - 51.85x + 53.61x^2 - 17.86x^3$, $R^2 = 0.95$). However, for the AR biotype, seedling emergence decreased sharply at depth > 0.5 cm ($y = 42.45 - 70.35x + 45.69x^2 - 9.63x^3$, $R^2 = 0.83$) at 30 d after sowing (Figure 4). Maximum emergence (93 and 47%) was achieved when seeds were placed on the soil surface for both the AS and AR biotypes of Asia Minor bluegrass, respectively. Decreased emergence with increased burial depth has been reported for many weed species (Bello et al. 2000; Fandrich and Mallory-Smith 2006; Rao et al. 2008). Asia Minor bluegrass seeds do not require light to germinate, but its seeds

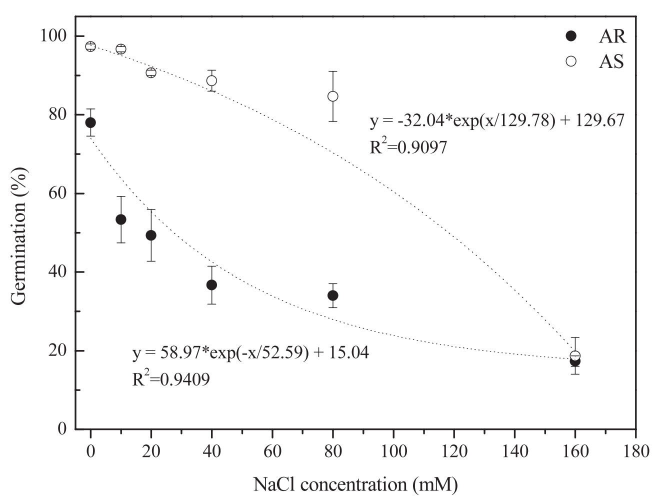

Figure 3. Effect of NaCl concentration on germination of aryloxyphenoxy propanoate herbicide-resistant (AR) and -susceptible (AS) Asia Minor bluegrass seeds at 15/5 C incubated with a 12-h photoperiod for 15 d. Vertical bars represent standard errors of the means.

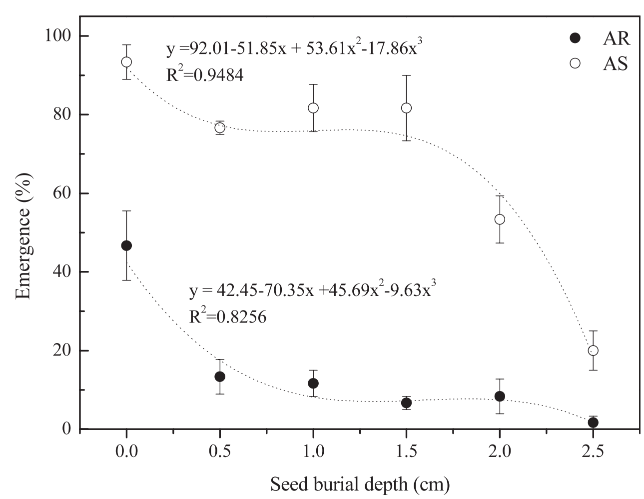

Figure 4. Effects of seed burial depth on seedling emergence of aryloxyphenoxy propanoate herbicide-resistant (AR) and -susceptible (AS) Asia minor bluegrass seeds in the greenhouse (30 d after sowing). Vertical bars represent standard errors of the means.

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are quite small and have limited ability to emerge from depth.

Asia Minor bluegrass can germinate at a wide pH range, osmotic potential, and NaCl concentration, and these factors are unlikely to be limiting factors for its further spread. However, germination of Asia Minor bluegrass is strongly affected by temperature and seed burial depth. The results indicated that this weed has the potential to become a problematic under no-till cropping systems. Management measures, such as control before December when temperature becomes unfavorable to germination and deep tillage, could effectively help farmers cope with this weed.

It has been shown that weed seed germination is determined by complex factors, driven by both genetic determinants and the environment (Baskin and Baskin 1998; Nonogaki et al. 2010). Vila-Aiub et al. (2009, 2011) showed that alleles endowing herbicide resistance can have pleiotropic effects on the weed life cycle, and that these effects depend on the allele. The results of this study indicated that the AR biotype of Asia Minor bluegrass displayed much lower germination than the AS biotype under different environment conditions in that it germinated under a relatively narrow temperature range, was more sensitive to pH, osmotic, and salt stress, and had very low germination when seeds were buried.

Previous studies have suggested a possible link between resistance to ACCase-inhibiting herbicides and variation in seed germination, emergence, and/or survival in the soil (Menchari et al. 2008; Owen et al. 2011; Vila-Aiub et al. 2005; Wang et al. 2010). Studies evaluating several fitness-related traits in blackgrass (*Alopecurus myosuroides* Huds.) with the Ile-2041-Asn mutation have shown no physiological or ecological resistance costs (Delye et al. 2013; Menchari et al. 2008). In selfing species, resistance alleles can arise in a variety of genetic backgrounds and the magnitude and expression of fitness cost will vary between populations and between individuals (Paris et al. 2008). In this research the AR Asia Minor bluegrass biotype possessing the same Ile-2041-Asn resistance allele displayed a reduction in seed germination; however, whether or not this would occur in other genetic backgrounds remains to be investigated.

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